CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor } University Farm, St. Paul 1, Minn.

Editorial Board: J. S. Andrews, M. J. Blish, G. Garnatz, Majel M. MacMasters, Betty Sullivan, J. A. Shellenberger

CONTENTS

Page

Studies on the Relation of Bran Thickness to Millability of Some Pacific Northwest Wheats. *R. A. Larkin, M. M. MacMasters, M. J. Wolf, and C. E. Rist* 247

The Distribution of Thiamine and Riboflavin in Rice Grains; and of Thiamine in Parboiled Rice. *I. A. Simpson* 259

Polysaccharides of High-Amylose Corn. *William Doench, H. H. Kramer, and Roy L. Whistler* 270

Utilization of Fungal Amylase for Alcohol Production. *Julian Corman and H. M. Tsuchiya* 280

The Distribution of Thiamine and Riboflavin in the Wheat Kernel at Different Stages of Maturity. *J. Marshall Pollock and W. F. Geddes* 289

Modification of the Buhler Mill for Micro Milling. *E. F. Seeborg, N. H. Shoup, and M. A. Barmore* 299

Amylose Content of Sweetpotato Starch. *Gordon L. Doremus, Frank A. Crenshaw and F. H. Thurber* 308

Loss of DDT Residues in Box Cars Carrying Flour. *B. Berck and B. N. Smallman* 317

Denaturation of Soybean Protein with Alcohols and with Acetone. *A. K. Smith, Vernon L. Johnsen, and Robert E. Derges* 325

Physiological Effects of Distillers' Dried Grains Derived from Acid-Saccharified Corn Mash. *Anthony M. Ambrose* 334

The Amino Acids of the Water Extract of Flour. *Betty Sullivan and W. E. Payne* 340

A Rapid Method for Determining the Viability of Barley. *Paul R. Gustafson* 343

Book Reviews 347

Editorial Policy and Suggestions to Authors 350

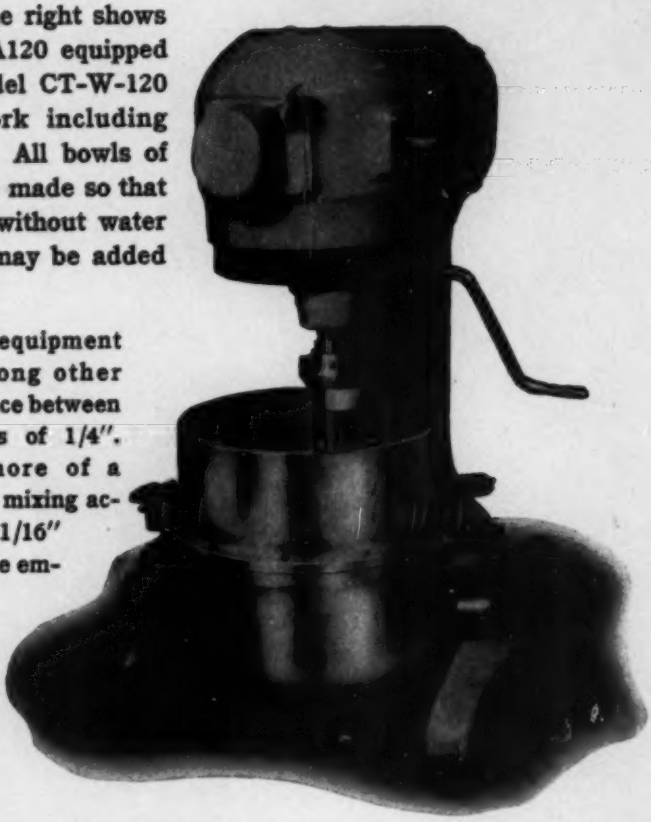
Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1924.

Vol. 28 July, 1951 No. 4

MIXING ATTACHMENTS

for your Hobart

The cut to the right shows the Hobart #A120 equipped with our Model CT-W-120 Bowl and Fork including water jacket. All bowls of this series are made so that if purchased without water jacket same may be added at any time.



This mixing equipment provides, among other things, a clearance between mixing surfaces of 1/4". This gives more of a STRETCHING mixing action than the 1/16" to 1/8" clearance employed in the Model known as the "McDuffee Bowl and Fork" which we have made in recent years.

For HOBART A200	Model CT-W-200	Water Jacketed Bowl, Stainless Fork	$95.00
For HOBART A200	Model CT-200	Plain Bowl, Stainless Fork	$65.00
For HOBART A120	Model CT-W-120	Water Jacketed Bowl, Stainless Fork	$90.00
For HOBART A120	Model CT-120	Plain Bowl, Stainless Fork	$60.00
For HOBART C10	Model CT-W-10	Water Jacketed Bowl, Stainless Fork	$80.00
For HOBART C10	Model CT-10	Plain Bowl, Stainless Fork	$50.00

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY

LINCOLN NEBRASKA

Good white bread made better...

THERE'S GOOD REASON why the Tenth Anniversary of bread and flour enrichment is being celebrated this year . . . because you have made good white flour and popular white bread better by enriching . . . and because this has meant a major contribution to the public's good health.

Be proud that you serve so well. By keeping faith with nature you make good foods better!

VITAMIN DIVISION • **HOFFMANN-LA ROCHE INC.** • NUTLEY 10, N. J.

In Canada: Hoffmann-La Roche, Ltd., Montreal, Que.

ROCHE

VITAMINS FOR ENRICHMENT

Keeping faith with nature

THE BRABENDER "LANGUAGE"

of flour characteristics has proven a most desirable help in the blending of wheat and the milling and selling of flour.

The FARINOGRAPH determines the type and basic strength of the wheat, its mixing requirements, its mixing tolerance, the relative absorption of the flour.

The EXTENSOGRAPH measures extensibility and resist ance to extension in the dough—two factors which determine the inherent volume potentialities of a flour, as well as maturing requirements, relative fermentation time and fermentation tolerance.

The AMYLOGRAPH brings out the malting requirements of the flour, for best crumb and texture.

A flour mill equipped with these three apparatus will be in a much better competitive position because of better wheat selection and better wheat blending, better maturing control, better malt dosing, generally improved control of flour uniformity, and a more foolproof means of making special flours for special purposes.

The acquisition of these apparatus is made very simple. They can be had on nominal monthly payments, all of them to be applied to the purchase price. In addition, there is the privilege to return the instruments at any time and for any reasons whatsoever and without further obligation.

You really can't afford *not* to avail yourselves of this modern "language".

BRABENDER CORPORATION, Rochelle Park, N.J.

THE NATIONAL LIFE LINE

A LINE OF ESSENTIAL BAKERY PRODUCTS

—supplies NATIONAL YEAST —uniform and reliable

NATIONAL brings you yeast of the highest purity that is uniform and reliable, so that you as bakers maintain even quality in the staff of life. NATIONAL also brings you a whole line of essential bakery products: NATIONAL YEAST FOOD, ACTIVE DRY YEAST, BAKING POWDER, MALT SYRUP and others, as well as:

Armour's Cloverbloom Frozen Whole Eggs

Armour's Cloverbloom Frozen Egg Whites

Armour's Cloverbloom Frozen Sugared Yolks

Armour's Dri Puff Paste

Armour's Armtex—the fortified egg

The NATIONAL Life Line is a line of supply —of bakery products and *dependable door to door services* that aid you in maintaining quality and uniformity in your baking, so your operations may continue successful and profitable.

Many of the largest and thousands of the smallest bakeries have been NATIONAL customers for years—a tribute to the service and uniform quality of NATIONAL products.

NATIONAL YEAST CORPORATION

FRANK J. HALL, President

EXECUTIVE OFFICES:
Chanin Building
New York, N. Y.

EASTERN DIVISION OFFICE:
45-04 27th Street
Long Island City, N. Y.

WESTERN DIVISION OFFICE:
35 E. Wacker Drive
Chicago, Ill.

PLANTS:
Belleville, N. J.
Crystal Lake, Ill.

DOUBLE ASSURANCE of Clean Grain *and* Highest Quality Products . . .

The "ENTOLETER" Scourer-Aspirator is the latest improvement in mill equipment for dry cleaning grain.

This equipment cleans grain in addition to the normal "ENTOLETER" function of destroying all forms of insect life. It eliminates insect fragments, rodent excreta and other contamination. After the grain passes through the "ENTOLETER" Insect Destroyer, it is thoroughly scoured in a whirling "tornado" action, and receives uniform aspiration at the most vital point.

While the light debris is in a state of suspension, dislodged from the grain, it is caught by suction and carried into the dust collector and on to feed.

Write for test data indicating the effectiveness of this equipment in actual mill operation. ENTOLETER DIVISION, The Safety Car Heating and Lighting Co., Inc., 1153 Dixwell Ave., New Haven 4, Conn.

ENTOLETER
CENTRIFUGAL MACHINES

CONTINUOUS INSECT CONTROL SYSTEM

Saves Time!
Prevents Waste!
Economical!
Always Uniform!

MILL CREEK **D-C** DIGESTION CATALYST **Tabs**

For Nitrogen Determinations In Kjeldahl Tests

KJELDAHL-WILFARTH-GUNNING METHOD

D-C TAB No. 3

$HgO + K_2SO_4$

Two Tablets supplies the catalyst ingredient and K_2SO_4 in the quantity and proportion as specified by the A.O.A.C. for the analysis of protein by the official Kjeldahl-Wilfarth-Gunning Method. (Formerly K-G-A)

GUNNING METHOD

D-C TAB No. 2

$CuSO_4 + K_2SO_4$

One Tablet supplies the catalyst ingredient and K_2SO_4 in the quantity and proportion as specified by the A.O. A.C. for the analysis of protein by the official Gunning Method.

Write For Samples

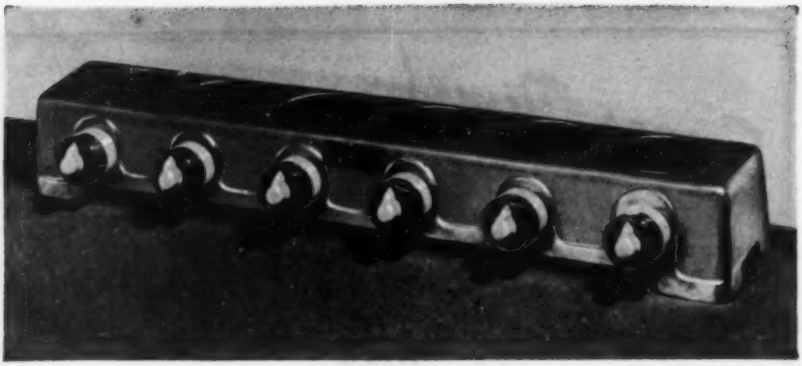
ON LABORATORY LETTERHEAD

ADVERTISED IN THE AMERICAN MEDICAL ASSOCIATION PUBLICATIONS

ADVERTISED IN THE Journal of the Association of Official Agricultural Chemists

Produced under the supervision of a pharmaceutical chemist. Each batch is analytically tested and recorded for uniform results.

D-C Tabs are packaged in standard containers of 100, 500 and 1000. Also in special lots of 5000.



SARGENT HOT PLATE

Evaporations, Digestions Crude Fiber Determinations Extractions With Soxhlet Apparatus

The six-place Sargent Hot Plate is tapped to accommodate seven support rods ½"x27" for use with Crude Fiber and Soxhlet assemblies. The solid disc heaters are individually controlled by separate switches (3 heat). Cool switching is assured, for the heaters are mounted on porcelain spools and have no direct contact with the base. The perforated bottom baffle plate acts as a heat reflector, eliminating scorching under the hot plate. The base itself is of cast aluminum. Dimensions: length, 28½"; width, 5¾"; height, 4".

S-41315 HOT PLATE — Electric, Sargent. For 115 volt AC/DC circuits. **$85.00**

S-31870 CRUDE FIBER ASSEMBLY —Six-Place, Electric, Sargent. Consisting of one **S-41315** Hot Plate; seven rods of stainless steel, ½" x 27" with 3/8-16 thread; six **S-4705** beakers, high form, without pourout, 600 ml., Pyrex; six **S-22742** condensers, reflexed hemisphere, Sargent; two **S-31872** clamps, water connector; five **S-31873** clamps, condenser support; rubber tubing. For 115 volt AC/DC circuits.....**$125.00**

S-31765 EXTRACTION ASSEMBLY —Six-Place, Large, Electric, Sargent. For medium and large Soxhlet assemblies, consisting of one **S-41315** Hot Plate; six rods of stainless steel, ½"x27" with ⅜" thread; 12 **S-31769** clamps, size B, spring, rubber covered. Less glassware. For 115 volt AC/DC circuits **$120.00**

S-31767 EXTRACTION ASSEMBLY —Six-Place, Small, Electric, Sargent. For small Soxhlet assemblies, consisting of one **S-41315** Hot Plate; six rods of stainless steel, ½" x 27" with ⅜" thread; 12 **S-31769** clamps, size A, spring, rubber covered. Less glassware. For 115 volt AC/DC circuits....**$120.00**

SARGENT

SCIENTIFIC LABORATORY INSTRUMENTS · APPARATUS · CHEMICALS

E. H. SARGENT & COMPANY, 4647 W. FOSTER AVE., CHICAGO 30, ILLINOIS

MICHIGAN DIVISION, 1959 EAST JEFFERSON STREET, DETROIT 7, MICHIGAN

SOUTHWESTERN DIVISION, 5915 PEELER STREET, DALLAS 9, TEXAS

SCIENTIFICALLY PREPARED FOR EXACTING USES

Always Uniform . . . Always Dependable

Yes, all-vegetable, all-hydrogenated COVO shortenings are made to specifications for exacting uses. There is one best suited to your particular needs.

COVO

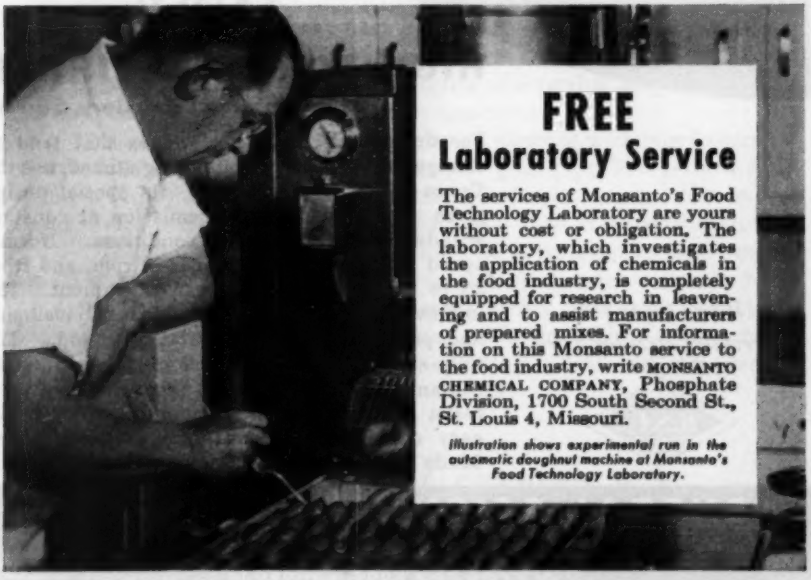
—the outstanding all-vegetable shortening for fine cakes, cookies, piecrust and fried foods. Covo is highly stabilized for long frying life.

COVO "S.S."

—the special shortening for prepared mixes—for biscuit and cracker manufacture, and for all other products requiring extra stability for long shelf life.

Covo *Shortenings*

LEVER BROTHERS COMPANY • GENERAL OFFICES: NEW YORK, N.Y.



FREE
Laboratory Service

The services of Monsanto's Food Technology Laboratory are yours without cost or obligation. The laboratory, which investigates the application of chemicals in the food industry, is completely equipped for research in leavening and to assist manufacturers of prepared mixes. For information on this Monsanto service to the food industry, write MONSANTO CHEMICAL COMPANY, Phosphate Division, 1700 South Second St., St. Louis 4, Missouri.

Illustration shows experimental run in the automatic doughnut machine at Monsanto's Food Technology Laboratory.

CENCO

HIGH-TORQUE STIRRER

. . . the best for Laboratory Stirring

For dependable stirring of mixes that tend to change in consistency while being stirred, use the Cenco High-Torque Stirrer. Its special design gives maximum power transmission at constant speeds under varying load conditions. Normal speed range is from 100 to 1300 rpm, and from 25 to 325 rpm with the gear attachment The improved chuck assures "hold fast" clamping and perfect aligning of the stirring rod. The fully-enclosed variable-speed mechanism avoids contamination of the mix. Stainless steel parts resist corrosion. Sturdy mounting rod permits easy clamping and positioning of the stirrer. Made for 115 or 230 volts AC or DC.

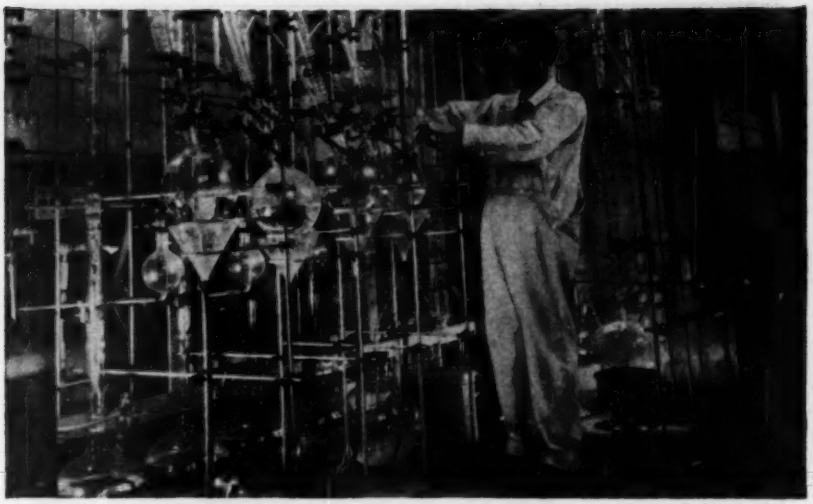
Write today for descriptive circular 1199.

R.S.C.

CENTRAL SCIENTIFIC COMPANY

Scientific Instruments • Laboratory Supplies

1700 Irving Park Road, Chicago 13.



PYREX brand glass apparatus for your larger process requirements

With the growing shortage of many non-corrosive materials, you may find the answer to your pilot plant or plant processing problem in large Pyrex brand glass apparatus. Heavy duty Pyrex flasks, for example, are made in sizes of 22, 50 and 72 liters. Large size cylinders are available in a variety of sizes; rugged 5 and 12 gallon Pyrex bottles offer further adaptation along with condensers, stop cocks and other apparatus.

Outlets may be provided with ground joints or with pipe flanges for connection to Pyrex brand "Double-Tough" glass pipe. This permits complete all glass assemblies for maximum corrosion resistance. Most important, Pyrex brand glass apparatus is available—and at reasonable cost.

Tough durable Pyrex brand glass 7740 is balanced to assure maximum service. Its chemical stability plus its high thermal resistance make it useful for a variety of purposes. Its transparency permits a visual check of every production run. Large size apparatus fabricated by Corning craftsmen and scientifically annealed may be a practical solution to your processing requirements. Why not look into it today?

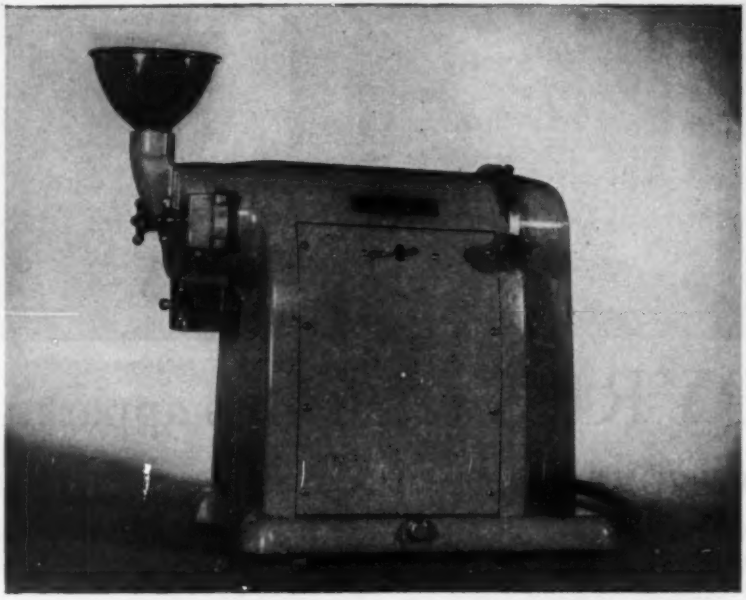
CORNING GLASS WORKS PYREX **CORNING, N. Y.**

Corning means research in Glass

1851 • 100 Years of Making Glass Better and More Useful • 1951

The New

"LABCONCO" Laboratory Mills



Our friends, "the experts", tell us sample preparation is the "trouble maker" and "bottleneck" in many cereal or feed laboratories. Two new mills by "Labconco" are helping solve this problem.

The Standard Model (price $225.00) has a ½ H.P. motor—is designed for the average cereal laboratory. The Heavy Duty Model (price $388.50) has a 1 H.P. motor—is recommended for the larger cereal laboratory where use is more constant and feedstuffs and other samples must be prepared.

Both models feature *micrometer setting* for endless variation of particle size, simple cleaning, and a newly designed cutting plate which produces a *"cut"* and representative sample. A card will bring you full details, list of users, comments on adaptability for your problems.

Laboratory Construction Company

1113-1115 Holmes Street, Kansas City, Missouri, U.S.A.

CEREAL CHEMISTRY

VOL. XXVIII JULY, 1951 NO. 4

STUDIES ON THE RELATION OF BRAN THICKNESS TO MILLABILITY OF SOME PACIFIC NORTHWEST WHEATS [1,2]

R. A. LARKIN, M. M. MACMASTERS, M. J. WOLF, AND C. E. RIST [3]

ABSTRACT

Thickness of the bran layers, viz., outer pericarp, cross and tube cell layer, spermoderm, nucellar layer, and aleurone layer of seven wheat varieties from the Pacific Northwest was determined microscopically in an effort to obtain data that might be correlated with the general milling behavior of the wheats.

The outer pericarp was found to be thickest toward the brush end, and approximately uniform in thickness over the rest of the kernel. Pericarp thickness varied among kernels of the same variety. Significant differences between varieties were not clearly shown.

The cross and tube cell layer was thickest over the dorsal side of the kernel; over the rest of the kernel the tube cells were either crushed or absorbed. The difference in thickness of the cross and tube cell layer between varieties was significant.

Significant differences in thickness of the spermoderm were found between varieties and between regions of the kernel. The spermoderm was thickest in the cheeks and gradually became thinner toward the dorsal side; while along the long axis it was thicker at the brush end and gradually became thinner toward the germ.

In transections the nucellar layer was thickest on the cheeks and in longisections it was thickest at the brush end. Significant differences between varieties and between regions were found.

The thickness of the aleurone layer varied significantly between varieties. Less difference in thickness of adjacent aleurone cells was found in good-milling than in poor-milling varieties. There was no conclusive evidence that this is a major factor affecting milling quality.

The thickness of the layers of the bran was not directly related to the general milling behavior of the wheat varieties studied.

An inverse relationship between bran thickness and flour yields of wheats has been postulated for many years. Recently, the relatively

[1] Manuscript received October 27, 1950.
[2] Presented at the Tri-Section Meeting of the American Association of Cereal Chemists, Manhattan, Kansas, October 21, 1950.
[3] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

low flour yields of the 1950 wheat crop in the Southwest have been attributed to unusually thick bran (1, 2). Bates (3) found that five wheat varieties had brans of different thicknesses. Shellenberger and Morgenson (6) reported that varietal differences in thickness of the bran layers were greater than differences due either to location where grown or to seasonal variation. They later found (7) that these differences were not related to flour yield.

Factors other than flour yield are involved in millability (5). It is not known what effect bran thickness has upon these.

Fundamental knowledge of the relationship of bran thickness to millability of wheat is of prime importance to millers and plant breeders. If bran thickness can be used as a criterion of milling quality, plant breeders can select on this basis at an early stage in the breeding program. Poor milling varieties could thus consistently be replaced by those of better milling quality. The farmer would gain by having a more ready outlet for his wheat if it were always acceptable to the miller. Conversely, the miller would profit by assured ease of processing and high yield of flour. The present studies were, therefore, undertaken to determine the relationship between bran thickness and millability of wheat. This work was carried out in informal collaboration with the Western Wheat Quality Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, where milling characteristics are studied, and the Western Regional Research Laboratory, which is investigating the chemical composition of wheats.

Variation in thickness of the outer four layers of the bran of three varieties with widely different milling characteristics was determined between sides of a kernel, regions of a kernel, kernels, and varieties in a factorially designed experiment. In addition, the thickness of the aleurone of seven varieties was studied.

Materials and Methods

The wheat samples used were obtained from the Western Wheat Quality Laboratory, where their milling qualities had been studied. Five criteria, straight flour yield, patent flour yield, milling rate, flour ash and moisture after tempering, were used in evaluating milling quality (5). Elgin and Hymar were excellent; Baart, Marfed, and Triplet were good; Brevor was fair; and Rex was poor, as determined by the laboratory milling tests. These varieties were soft, with the exception of Triplet, Baart and Marfed which were semihard. Each sample was from a single plot. Kernels were prepared by steeping for one hour in distilled water at room temperature, sections 30μ thick were cut on the freezing microtome; and the sections were

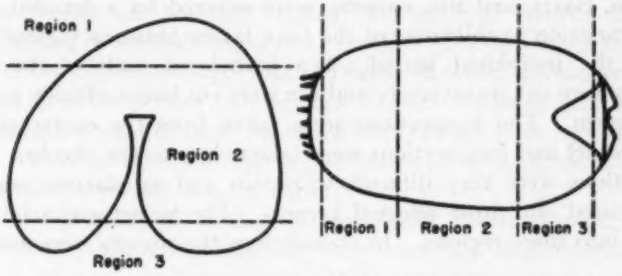


FIG. 1. The regions arbitrarily assigned to (left) transections and (right) longisections of the wheat kernel.

stained and mounted in a Congo Red solution buffered to pH 8 with phosphate buffer. Good differentiation between structures was obtained by use of this stain. This solution caused an increase in thickness of the bran of 0.54μ over that of sections mounted in water. The thickness of the aleurone was increased an average of 0.29μ in a similar study. However, these increases in thickness were within the limits of error of the measurements.

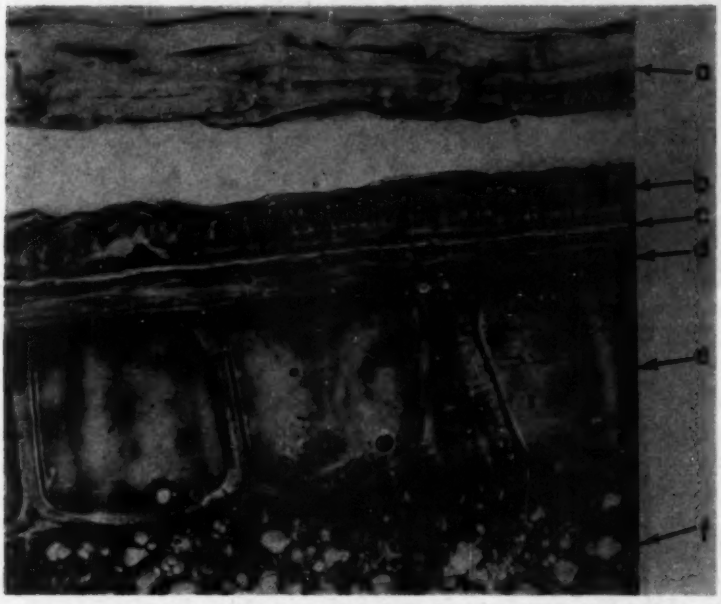


FIG. 2. Transection of Baart wheat showing the structure of the outer layers of the wheat kernel. (a) Outer pericarp, (b) cross cells, (c) spermoderm, (d) nucellar layer, (e) aleurone, (f) starchy endosperm. Magnification 500 ×.

Elgin, Baart, and Rex varieties were selected for a detailed study of the variation in thickness of the bran layers between varieties and around the individual kernel. Ten kernels of each of the three varieties were cut transversely and ten were cut longitudinally parallel to the germ. The transections were taken from the center portion of the kernel and longisections were taken close to the cheeks. True longisections were very difficult to obtain and satisfactory sections were secured only from selected kernels. The kernel was arbitrarily divided into three regions. In transections the regions were assigned

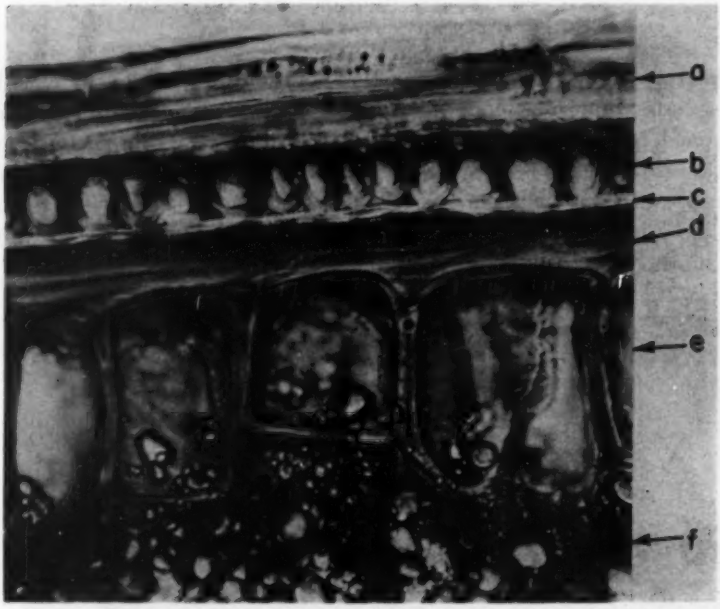


FIG. 3. Longisection of Baart wheat showing the structure of the outer layers of the wheat kernel. (a) Outer pericarp, (b) cross cells, (c) spermoderm, (d) nucellar layer, (e) aleurone, (f) starchy endosperm. Magnification 500 ×.

as shown in Fig. 1a; the crease was not considered in these studies. Regions in the longisections were assigned as indicated in Fig. 1b. Measurements of the thickness of the outer pericarp, cross and tube cell layer, spermoderm, and nucellar layer were made on each side of the kernel in each region, making a total of six determinations for each structure on each section. All of the aleurone cells on one side of transections and longisections, respectively, were measured. Typical views of the five structures in transections and longisections are given in Figs. 2 and 3, respectively.

The studies on Hymar, Marfed, Triplet, and Brevor varieties were confined to transections of five kernels of each variety. Measurements of the outer pericarp, cross and tube cell layer, spermoderm, and nucellar layer were made at one place in each region of each section, thus making a total of three measurements of each bran layer on each kernel. Fifteen aleurone cells were measured in each of the three regions on five kernels of each variety.

Statistical Analysis of Experimental Data. Only the data from Elgin, Baart, and Rex varieties were analyzed statistically. The "t" test (8) was used to analyze the measurements of the aleurone cells since the total number of measurements in each variety was different (transections, Elgin 1192, Baart 1087, and Rex 1219; longisections, Elgin 1268, Baart 1648, and Rex 1301). The 180 measurements, each, of the outer pericarp, cross and tube cell layer, spermoderm, and nucellar layer were subjected to the analysis of variance (4).

To study the significance of the variance of each variable, different error variances were used for the main effects and for the first order interactions. The second and third order interactions were pooled and used as the error variance of the experiment. This generalized error term was used to measure the significance of the first order interactions. The significance of the main effects was measured by either the most significant first order interaction in which it occurred, if it exceeded the 5% level, or by the generalized error term.

Results and Discussion

The analyses of variance of transections and longisections of Elgin, Baart, and Rex wheat varieties are summarized in Tables I and II, respectively. The variety means of transections and longisections of the three varieties are given in Table III, while the variety means for Brevor, Hymar, Triplet, and Marfed varieties are given in Table IV.

Outer Pericarp. The data on measurements of outer pericarp from transections and longisections are not in complete agreement. Elgin had the thickest outer pericarp in transections and thinnest in longisections, and the relative positions of Baart and Rex were reversed. In transections, the kernels within each variety varied so much that significant differences between varieties were not shown. In longisections, the regions within varieties did not follow the same trend; they were in the following sequence from thickest to thinnest: Elgin 1, 3, 2, Baart 1, 2, 3, and Rex 1, 3, 2. Even with this much variation, the difference in thickness between varieties and between region 1 and the other regions was large enough to be significant.

TABLE I

ANALYSIS OF VARIANCE—SHOWING THE DISTRIBUTION OF VARIANCE IN THICKNESS OF FOUR LAYERS OF THE BRAN IN TRANSECTIONS OF ELGIN, BAART, AND REX WHEAT VARIETIES

Source of Variance	Degrees of Freedom	Mean Squares			
		Outer Pericarp	Cross and Tube Cell Layer	Spermoderm	Nucellar Layer
Varieties	2	380.22	539.82	5.47**	153.61**
Kernels	9	156.78	22.36	0.19	13.22
Regions	2	14.82	1493.52*	1.62**	276.58**
Sides-of-a-kernel	1	0.02	7.61	0.16	1.15
Varieties × Kernels	18	129.48**	34.79	0.37*	13.26**
Varieties × Regions	4	42.66	123.33**	0.25	12.84*
Varieties × Sides-of-a-kernel	2	13.07	32.44	0.21	1.31
Kernels × Regions	18	17.88	23.08	0.15	5.30
Kernels × Sides-of-a-kernel	9	18.13	24.06	0.12	2.11
Regions × Sides-of-a-kernel	2	4.77	59.01	0.18	11.65*
Error	112	20.93	25.38	0.19	3.74

* Significant at 5% level.
** Significant at 1% level, or highly significant.

The mean thickness of the outer pericarp of Hymar, Triplet, Marfed, and Brevor showed differences between varieties but they were not related to the general milling behavior of the varieties.

Cross and Tube Cell Layer. Elgin had the thinnest layer of cross and tube cells, Baart the thickest, and Rex intermediate, in both transections and longisections. The regions did not vary in the

TABLE II

ANALYSIS OF VARIANCE—SHOWING THE DISTRIBUTION OF VARIANCE IN THICKNESS OF FOUR LAYERS OF THE BRAN IN LONGISECTIONS OF ELGIN, BAART, AND REX WHEAT VARIETIES

Source of Variance	Degrees of Freedom	Mean Squares			
		Outer Pericarp	Cross and Tube Cell Layer	Spermoderm	Nucellar Layer
Varieties	2	2111.51*	182.25**	7.39**	213.09*
Kernels	9	78.60	4.96	0.18	20.63
Regions	2	2412.82*	2.57	4.76**	165.75*
Sides-of-a-kernel	1	108.89	4.05	0.33	3.07
Varieties × Kernels	18	65.51*	4.06	0.23	9.95**
Varieties × Regions	4	263.56**	9.44*	0.06	15.04**
Varieties × Sides-of-a-kernel	2	83.24	0.08	0.05	4.51
Kernels × Regions	18	49.85	4.97	0.19	3.08
Kernels × Sides-of-a-kernel	9	36.65	5.36	0.32*	4.72
Regions × Sides-of-a-kernel	2	23.76	1.52	0.06	7.52
Error	112	36.97	3.32	0.13	4.02

* Significant at 5% level.
** Significant at 1% level, or highly significant.

same order within each variety. In transections they were arranged in the following sequence from thickest to thinnest: Elgin 1, 2, 3, Baart 1, 3, 2, and Rex 1, 3, 2, while in longisections they were: Elgin 1, 2, 3, Baart 2, 3, 1, and Rex 1, 3, 2. Even with so much variation in regions 2 and 3 of transections, region 1 was significantly thicker than the other regions. This was due in part to the unflattened nature of the tube cells which was observed only in this region. The difference between varieties, in longisections, was highly significant, even though the difference was not as large in longisections as in transections. This was because transections showed a greater variation between regions within varieties than did longisections.

TABLE III

THICKNESS OF BRAN LAYERS FROM TRANSECTIONS AND LONGISECTIONS OF ELGIN, BAART, AND REX WHEAT VARIETIES

Variety[1]	Mean Thickness				
	Outer Pericarp	Cross and Tube Cell Layer	Spermoderm	Nucellar Layer	Total
	μ	μ	μ	μ	μ
Transections					
Elgin	31.5	14.8	2.0	11.5	59.8
Baart	29.0	20.5	2.5	13.0	65.0
Rex	26.4	15.8	2.0	9.8	54.0
Longisections					
Elgin	26.2	13.4	2.2	14.5	56.2
Baart	38.1	16.6	2.8	14.0	71.5
Rex	32.6	13.9	2.3	11.0	59.8

[1] Arranged in order of decreasing milling quality.

The mean thickness of the cross and tube cell layer of Hymar, Triplet, Marfed, and Brevor did not have as wide a range as the means of Elgin, Baart, and Rex. The thicknesses, however, were not related to the general milling behavior of the varieties.

Spermoderm. The spermoderm, or seed coat as it is commonly known, is a very thin structure. It varied in thickness from 1.5 to 3.5μ over the different parts of the kernel, and differences between regions were highly significant. Region 3, i.e., the cheeks, had the thickest spermoderm, the sides intermediate, and region 1 the thinnest in transections. In longisections, the spermoderm was thickest at the brush end and gradually became thinner toward the germ. The

differences between varieties were highly significant in both transections and longisections, even though the kernels within each variety were different in transections. Baart had the thickest spermoderm and Rex and Elgin were approximately the same. The kernels × sides-of-a-kernel interaction was significant at the 5% level, which shows that the spermoderm at the sides of each kernel was not consistently of the same thickness.

The mean thickness of the spermoderm of all seven varieties did not appear to be related to the milling behavior of the varieties. It is interesting to note, however, that Rex and Brevor, the poorest milling wheats studied, had thin spermoderms while Hymar, an excellent milling wheat, had a thick spermoderm; Elgin was an exception.

Nucellar Layer. The thickness of the nucellar layer followed the same trend in transections and longisections. In transections the average thickness in microns was: Elgin 11.5, Baart 13.0, and Rex

TABLE IV

THICKNESS OF BRAN LAYERS FROM TRANSECTIONS OF HYMAR, TRIPLET, MARFED, AND BREVOR WHEAT VARIETIES

Variety[1]	Mean Thickness				
	Outer Pericarp	Cross and Tube Cell Layer	Spermoderm	Nucellar Layer	Total
	μ	μ	μ	μ	μ
Hymar	24.7	13.8	2.4	11.0	51.9
Triplet	30.3	18.2	2.5	13.9	64.9
Marfed	24.3	17.9	2.1	10.4	54.7
Brevor	29.3	14.4	2.1	11.1	56.9

[1] Arranged in order of decreasing milling quality.

9.8; and in longisections it was: Elgin 14.5, Baart 14.0, and Rex 11.0. The differences between varieties were highly significant in transections and significant in longisections. The kernels within each variety varied in thickness of nucellar layer in both transections and longisections. Variations in thickness of the nucellar layer of the regions within each variety were significant in transections and highly significant in longisections. Even though the regions within each variety varied, the differences between regions of all the kernels were highly significant in transections and significant in longisections. The nucellar layer in the cheeks was thicker than in the other regions in transections. In longisections this layer was thicker at the brush end than over the rest of the kernel. The analysis also indicated that the interaction, regions × sides-of-a-kernel, was significant in transections.

TABLE V

THICKNESS OF ALEURONE LAYER AND TOTAL NUMBER OF ALEURONE CELLS MEASURED ON SECTIONS OF THE SEVEN VARIETIES OF WHEAT

Variety[1]	Mean Thickness			
	Transections		Longisections	
	Mean Thickness	Number of Measurements	Mean Thickness	Number of Measurements
	μ		μ	
Elgin	46.0	1,192	46.0	1,268
Hymar	47.8	225	—	—
Triplet	54.7	225	—	—
Marfed	51.6	225	—	—
Baart	54.0	1,087	59.7	1,648
Brevor	45.3	225	—	—
Rex	51.4	1,219	53.6	1,301

[1] Arranged in order of decreasing milling quality.

Data on four additional varieties (Table IV) indicated differences between varieties, but these differences showed no correlation with the general milling behavior of the varieties.

Aleurone Layer. In Elgin, Baart, and Rex, the mean thickness of the aleurone layer was approximately the same in transections and longisections, but there was a greater difference between varieties in longisections than in transections (Table V). Elgin had the thinnest aleurone layer, Baart the thickest, and Rex was intermediate.

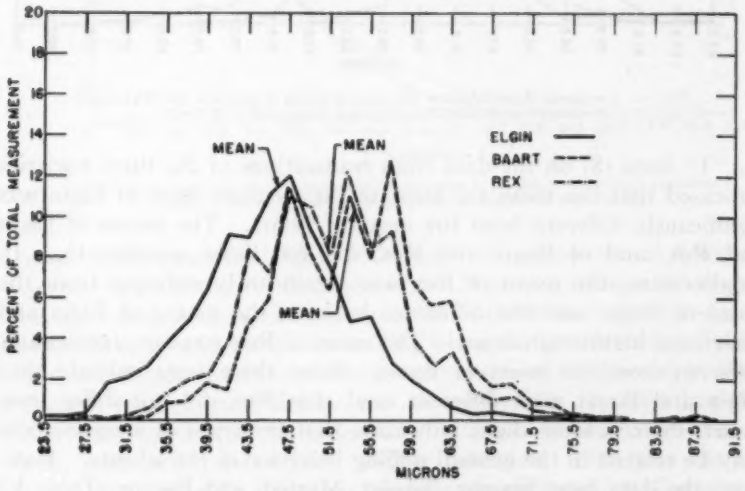


FIG. 4. Frequency distribution of the measurements of aleurone cell thicknesses in microns on cross sections of Elgin, Baart, and Rex wheat.

Frequency distributions of the thickness of the aleurone layer of the three varieties are given for transections and longisections in Figs. 4 and 5, respectively. The distribution in thickness of the aleurone layer in Elgin appeared to follow a normal curve rather closely, but Baart and Rex had irregular distributions near their means even though their general distribution followed a normal curve. Calculations on the original data show that in transections Elgin had a standard deviation of 8.16, Baart 7.70, and Rex 6.68; in longisections the standard deviations were: Elgin 6.61, Baart 9.37, and Rex 8.82. Elgin was most variable in transections and Baart most variable in longisections. Rex varied less in aleurone thickness than the other varieties.

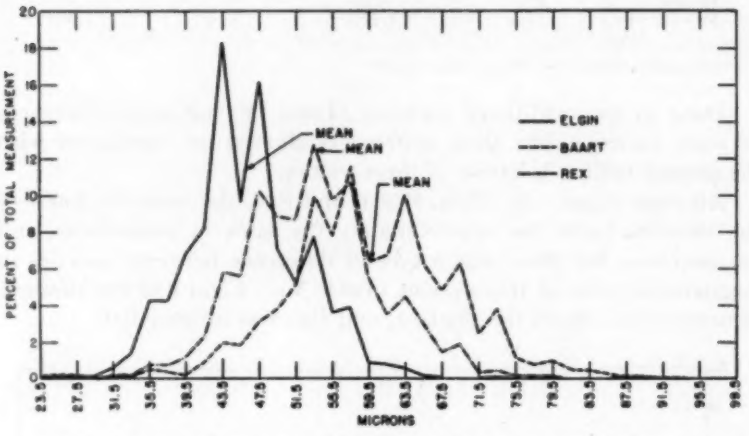


FIG. 5. Frequency distribution of the measurements of aleurone cell thicknesses in microns on longisections of Elgin, Baart, and Rex wheat.

"T" tests (8) on the data from transections of the three varieties indicated that the mean thickness of the aleurone layer of Elgin was significantly different from the mean of Baart. The means of Elgin and Rex, and of Baart and Rex, did not differ significantly. In longisections, the mean of Rex was significantly different from the mean of Elgin, and the difference between the means of Elgin and Baart was highly significant. The mean of Rex was not significantly different from the mean of Baart. Since these tests indicate that Elgin and Baart were different, and that Rex did not differ from Baart, there is some slight indication that thickness of aleurone cells may be related to the general milling behavior of the wheats. However, the data from Hymar, Triplet, Marfed, and Brevor (Table V) do not confirm this theory. Brevor, a fair milling wheat, had a thinner

aleurone layer than either Elgin or Hymar, both excellent milling wheats.

In an attempt to determine the manner in which the aleurone cells varied in thickness, the difference in thickness between adjacent aleurone cells was calculated. Frequency distributions of these calculations from longisections of Elgin and Rex are presented in Fig. 6. Elgin had a larger number of differences between adjacent aleurone cells concentrated in the 1.0 to 2.0μ range than Rex, but the rest of the distribution for the two varieties was very similar. The other five varieties followed this same general pattern. Hymar

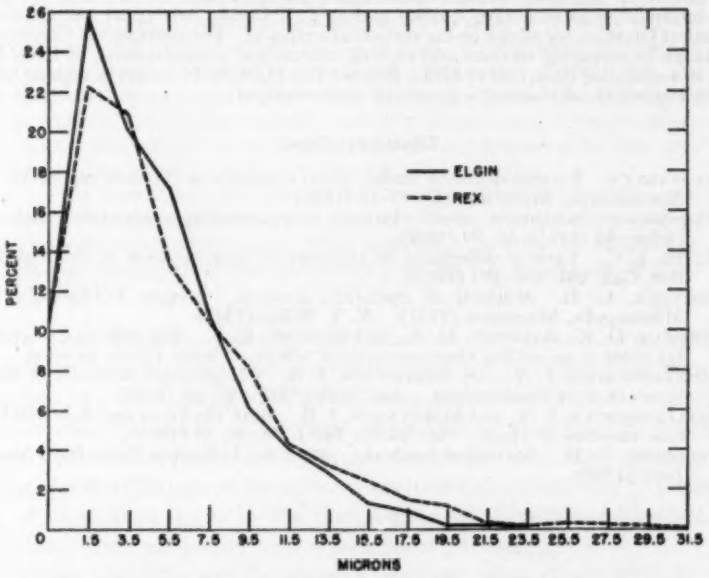


FIG. 6. Frequency distribution of the differences in thickness in microns between adjacent aleurone cells of longisections of Elgin and Rex wheat.

most closely resembled Elgin in having a large number of differences concentrated in the 1.0 to 2.0μ range. These data indicated that the better milling wheats had less variation in thickness between adjacent aleurone cells than the poorer milling wheats. However, the distribution of differences between adjacent aleurone cell thicknesses in Baart, Triplet, Marfed, and Brevor was so nearly like the distribution in Rex that there was no apparent distinction between the varieties with poor and intermediate milling behavior. This was the only factor considered in these studies that showed any correlation with milling data. However, differences between intermediate and poor

milling wheat varieties could not be distinguished by the methods used in these studies.

It seems improbable on the basis of the present data that the mean thickness of the bran or of any of its layers can be used as an indication of the milling quality of a wheat variety. Further work will be needed to determine whether a correlation exists between the variation in thickness of the aleurone layer within the kernel and the millability of the wheat.

Acknowledgment

The authors are indebted to Mr. Dale K. Mecham of the Western Regional Research Laboratory for drawing attention to the urgency of this study, to Mr. Mecham and Dr. M. A. Barmore, Western Wheat Quality Laboratory, for the wheat samples and information as to milling quality and to J. J. Curtis, Analytical and Physical Chemical Division, for advice on the statistical analyses. The assistance of Constance L. Buzan in preparing sections and making microscopic measurements, of Irene M. Cull in assembling data, and of Helen Bowker and Marjorie J. Austin in making part of the statistical calculations is gratefully acknowledged.

Literature Cited

1. ANONYMOUS. Further details of earlier wheat character in chemists report No. 2. Northwestern Miller **243** (1): 12–13 (1950).
2. ANONYMOUS. Southwest cereal chemists crop reporting committee. Bakers' Helper **94** (1173): 60, 99 (1950).
3. BATES, J. C. Varietal differences in anatomy of cross-sections of wheat grain. Bot. Gaz. **104**: 490–493 (1943).
4. GOULDEN, C. H. Methods of statistical analysis. Burgess Publishing Co., Minneapolis, Minnesota (1937). N. Y. Wiley (1939).
5. MECHAM, D. K., BARMORE, M. A., and SEEBORG, E. F. The influence of crude fat content on milling characteristics of wheat. Cereal Chem. in press.
6. SHELLENBERGER, J. A., and MORGENSON, J. B. Comparative thickness of bran layers of hard winter wheat. Am. Miller **77**(8): 37, 40 (1949).
7. SHELLENBERGER, J. A., and MORGENSON, J. B. Bran thickness and flour yield of four varieties of wheat. Am. Miller **78**(8): 29, 30, 38 (1950).
8. SNEDECOR, G. W. Statistical methods. 4th Ed. Collegiate Press Inc., Ames, Iowa (1946).

THE DISTRIBUTION OF THIAMINE AND RIBOFLAVIN IN RICE GRAINS; AND OF THIAMINE IN PARBOILED RICE [1]

I. A. SIMPSON[2]

ABSTRACT

The distribution of thiamine and riboflavin in rice grains has been studied by the photographic method, first developed by Somers and his colleagues, for locating these vitamins in wheat kernels; some examples of the photographs obtained are presented. The results confirm that the maximum concentration of both thiamine and riboflavin occurs in the germ or embryo; but, whereas thiamine is largely concentrated in the scutellum and the adjacent cells of the endosperm, riboflavin appears to be more uniformly distributed throughout the tissues of the embryo.

The method has also been applied to observe the changes that occur in the distribution of thiamine in rice grains during the parboiling process. The results show that, under certain conditions, considerable diffusion of thiamine from the scutellum and pericarp tissues into the endosperm takes place.

In 1945, Somers, Coolidge and Hamner (14) described a simple and ingenious method for demonstrating, by photographic means, the location of thiamine and riboflavin in wheat kernels. During the course of a study of methods of parboiling rice now in progress in this laboratory, this photographic technique has been applied to locate the distribution of these vitamins in the rice grain and to observe the changes that occur in the distribution of thiamine in rice grains that have been subjected to the process of parboiling

Altson and Simpson (2) showed, in 1941, that about 50% of the total content of thiamine in the rice grain is concentrated in the germ or embryo, which only comprises 2% of the weight of the grain. More recently, Hinton (7) has examined in detail the distribution of thiamine in the rice grain by dissecting out the various parts and analyzing them. He found that 44–50% of the total thiamine is located in the scutellum, 34–35% in the pericarp, aleurone layers and the covering of the germ, 7–9% in the endosperm and the remainder (9–12%) in the remaining parts of the embryo.

During the process of milling, the greater part of the pericarp and aleurone layers, together with the germ (including the scutellum), is

[1] Manuscript received November 22, 1950.
[2] Biochemist, Institute for Medical Research, Kuala Lumpur, Federation of Malaya.

removed in the "polishings" or bran. As a result, highly milled rice contains only a small proportion of the thiamine originally present in the grain and is thus liable to cause beriberi when consumed as a dietary staple. It has long been known that the substitution of milled, parboiled rice in place of highly milled, untreated rice will prevent this risk, since parboiled rice, even when highly milled, retains 50–70% of the thiamine present in the original padi. It has been generally accepted that this is due to a redistribution of the water-soluble nutrients (including thiamine) of the grain, so that the endosperm is enriched at the expense of the integuments (3, 15). However, Nicholls (10) has observed that, in contrast to untreated rice, most samples of parboiled rice that he had examined, appeared to retain the scutellum portion of the germ after milling; and he suggested that the nutritional advantage of milled, parboiled rice over milled, untreated rice might be explained by this fact, rather than by the redistribution of nutrients. By applying the technique of dissection and analysis of the separated tissues to samples of parboiled rice, Hinton (8) has shown that absorption of thiamine by the endosperm does occur, under certain conditions, during the parboiling process; and he has also shown that gelatinization of the cells of the endosperm contiguous to the scutellum, accompanied by toughening of the intervening membrane, occurs during the heating process. As a result of these changes, the scutellum becomes more adherent to the endosperm and is less liable to be removed during milling than in the case of untreated rice.

The work now presented was undertaken to find out if visual confirmation of these changes could be obtained by the photographic technique and to observe, if possible, to what extent the absorption of thiamine into the endosperm is affected when the conditions of parboiling are varied.

Materials and Methods

As far as possible, the method described by Somers *et al.* (*loc. cit.*) has been followed. A Leica IIc camera, with Leitz Elmar 5 cm. lens and fitted with an extension tube to give a direct magnification of approximately three diameters, was used. In place of Corning filters, the following Wratten filters were used and found to be satisfactory: for thiamine, No. 18A in front of the mercury vapor lamp and Nos. 8 and 39 on the camera; and for riboflavin, No. 36 on the lamp and Nos. 2A and 21 in front of the camera.

Kodak Plus X film was used for recording the thiamine (thiochrome) fluorescence, but, owing to the greatly increased exposures required to record the fluorescence, Kodak Super XX film was found

to be preferable for riboflavin. In each case, the film was developed with Kodak D76 developer and the prints made on high contrast printing paper. It was also found to be advantageous to concentrate the beam of filtered ultra-violet radiation on the subject by means of a quartz lens of suitable focal length.

In order to ensure that these filter combinations transmitted specifically the fluorescent light characteristic, respectively, of thiamine and of riboflavin, photographs were taken of a series of test strips of filter paper (approximately 2.5 cm. × 1.5 cm. in size), which had been treated in the manner described by Somers. The results obtained are shown in Fig. 1. No sample of co-carboxylase was available for testing, but Somers records that the method will not distinguish

FIG. 1. Photographs of treated filter paper strips. *Left* (Group A). Obtained by using filters selective for thiochrome fluorescence, i.e., Wratten filters No. 18A in front of the ultra-violet lamp and Wratten filters Nos. 8 and 39 on the camera. *Right* (Group B). Obtained by using filters selective for riboflavin fluorescence, i.e., Wratten filter No. 36 in front of the ultra-violet lamp and Wratten filters Nos. 2A and 21 on the camera. The strips in both groups were treated as follows: Top row, left to right. Control; thiamine; riboflavin. Bottom row. Same as top row, except that all strips have been treated with an alkaline ferricyanide solution.

between co-carboxylase and thiamine. No distinction is made between these two substances in this work.

In order to obtain a light background, several samples of local clay were tested, but none was found to fluoresce when illuminated by ultra-violet radiation; and it was necessary to use a piece of card, impregnated with a mixture of paraffin wax and a heavy mineral oil, as a background for the strips. This was not, however, suitable for mounting the rice grains.

The results of photographing the test strips using the filter combinations described show that they record, specifically, the fluorescence excited, respectively, by thiochrome and riboflavin when illuminated by ultra-violet radiation of suitable wave-length. The control strips

were plain, untreated filter paper. The vitamins were applied to the respective filter paper strips in amounts equal, approximately, to 0.01 ml. of a millimolar solution per square centimeter; and are thus present in each of the treated strips in approximately comparable concentrations. Those that were treated with alkaline ferricyanide solution were all treated alike, after they had been dried in the dark, with a mixture of 5 parts of 5% sodium hydroxide and 1 part of 0.5% potassium ferricyanide solutions. They were dried in the dark before they were photographed. It is noted that, with the filter combination used for thiamine, some riboflavin fluorescence is recorded (Fig. 1, Group A—right hand strip, top row), but this disappears when the riboflavin strip had been treated with alkaline ferricyanide (Fig. 1, Group A—right hand strip, bottom row). It was concluded, therefore, that under the conditions used no interference by riboflavin would occur when photographing the thiochrome fluorescence exhibited in rice sections.

Preparation of Rice Sections. With the exception of the samples of commercially prepared parboiled rice, which were purchased from local shops, all the rice used was hulled and milled in this laboratory from one batch of padi grown in the Tanjong Karang district of Selangor, Federation of Malaya. Photographs have been taken of a large number of grains of padi, hulled rice, and milled rice. Those selected for illustration are of hulled rice, which is considered to be the most suitable for demonstrating the location of thiamine and riboflavin in the grain.

The grains were cut by hand with a razor to expose, as far as possible, the broadest surface of the section required. Owing to the consistency of the grains, this was not always easy to effect, particularly in the case of the parboiled samples. In the majority of cases, the following sections of the grain were cut and photographed: (*a*) Broad, longitudinal section. (*b*) Narrow, longitudinal section. (*c*) Cross section at the middle of the grain. (*d*) Cross section at the germ end of the grain.

The sectioned grain, cut surface uppermost, was mounted on a thin layer of plasticine spread on a small board. The plasticine exhibited no fluorescence under ultra-violet radiation and the background of the photographs is, therefore, dark. To some extent, this is an advantage, since it shows more clearly the somewhat faint fluorescence derived from the vitamins in the pericarp and aleurone layers.

In the case of sections cut for thiamine, it was found preferable to apply the alkaline ferricyanide solution lightly to the cut surface with a camel's-hair brush. If the solution is applied as a drop, the photograph

is more liable to be blurred, possibly due to diffusion of the thiamine in the rice tissues caused by excess liquid. The sections were dried in the dark before being photographed.

Results and Discussion

Thiamine in Untreated, Raw Rice. When photographs were taken, using the thiamine filter combination, of sections which had not been treated with alkaline ferricyanide, only a very faint fluorescence was recorded; this may be due to the presence of traces of fluorescent material, other than thiamine or riboflavin, in the grain. Since a non-fluorescent background was used, this very faint fluorescence does not show up clearly in photographs and no examples are, therefore, shown.

Examples of the results obtained when sections that had been treated with alkaline ferricyanide were photographed using the filters selective for thiamine, are shown in Fig. 2. Considerable variation has been found in the intensity of the fluorescence derived from thiamine in different, individual grains, but in all cases it has been found, as was to be expected, that the maximum concentration of thiamine occurs in the scutellum and in the cells of the endosperm adjacent to the embryo. The remainder of the embryo, other than the scutellum, does not appear to be rich in thiamine. A thin layer of cells (pericarp and aleurone layers) surrounding the endosperm also shows the presence of thiamine, but the amount seems to be variable. Photographs of some grains show a more or less continuous band of thiamine-containing cells surrounding the endosperm, but in other cases, this fluorescent band is either partially or entirely absent, in spite of the fact that the pericarp layers appeared to be intact.

"Chalky" Grains. In many cases, considerable areas of the endosperm have shown marked fluorescence and two examples of such grains are shown in Fig. 3. Comparison of the photograph with the cut surface of the grain photographed has shown that these areas—which presumably contain thiamine—correspond with areas in the grain that are opaque and "chalky" in appearance. It is known that these opaque, "chalky" areas are found in grains that have become affected by bacterial action; and aerobic, spore-forming bacilli, such as *B. vulgatus*, have been isolated from the interior of such grains (1, 6, 13). Many years ago, this group of bacilli was reported as possessing the ability to synthesize vitamin B_1 (11, 12). The present work appears to offer some support to this observation and further investigation of this point is in progress. Attention has, however, been drawn to this at the present stage, since it must be borne in mind when photographs of parboiled rice are examined, in order to

distinguish between the fluorescence of these areas and that due to thiamine that may have diffused into the endosperm as a result of the parboiling process.

Riboflavin in Untreated, Raw Rice. Examples of the photographs of hulled, raw rice, using the filter combination selective for riboflavin, are shown in Fig. 4. It has, unfortunately, not been found possible

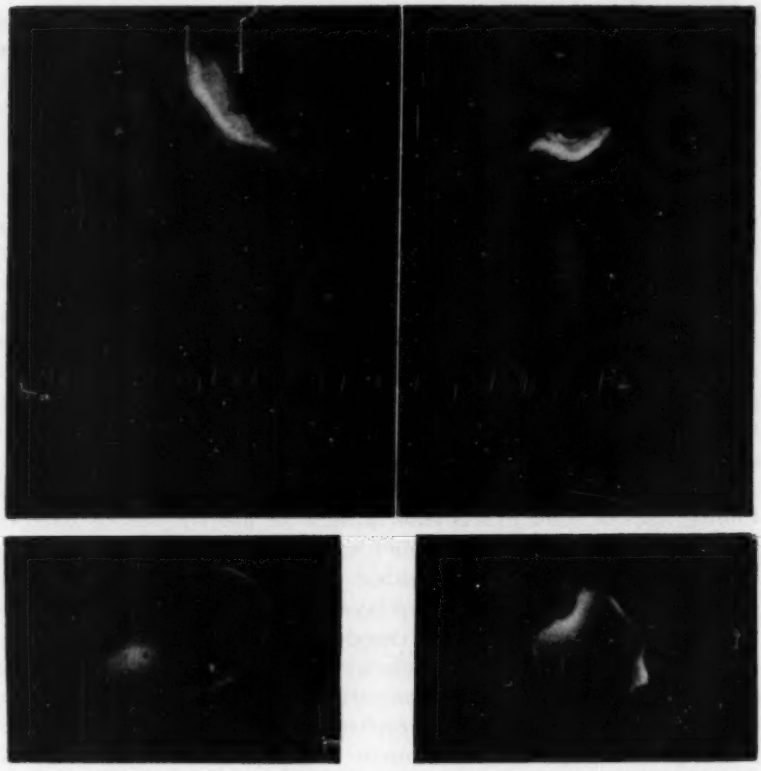


FIG. 2. Location of Thiamine in hulled rice. *Top left.* Broad longitudinal section. *Top right.* Narrow longitudinal section. *Bottom left.* Cross section, center of grain. *Bottom right.* Cross section at germ end of grain. Magnification: approx. 10 X.

to obtain as sharp a definition as is obtained when the filters selective for thiamine are used. Nevertheless, these photographs indicate that riboflavin is also largely concentrated in the embryo; but seems to be more evenly distributed throughout the tissues of the embryo than is the case with thiamine.

Thiamine in Parboiled Rice. The usual, classical method of parboiling rice as practised in India and other Asian countries consists

FIG. 3. Location of Thiamine in hulled rice. Examples of "chalky" grains. Broad longitudinal sections. Magnification: approx. 10 ×.

of soaking the padi in cold water for one or two days, or even longer, followed by steaming at atmospheric pressure for about thirty minutes, after which the treated padi is sun-dried, hulled and milled. The resulting, milled, parboiled rice consists of semi-translucent grains that vary in color from cream to yellow and which possess a flavor

TABLE I

THIAMINE CONTENT OF HIGHLY MILLED RICE DERIVED FROM PADI SUBJECTED TO DIFFERENT TREATMENTS

Period of Soaking	Methods of Treatment of the Padi		
	Cold Water Soak Not Autoclaved[1]	Cold Water Soak Autoclaved[2]	Hot Water Soak Autoclaved[3]
	μg./g.	μg./g.	μg./g.
Nil. Autoclaved only	—	—	2.00
5 minutes	—	—	2.53
15 minutes	—	—	2.31
30 minutes	—	—	2.34
1 hour	0.75	2.26	2.15
2 hours	—	—	2.34
3 hours	0.60	2.40	2.23
6 hours	0.76	2.56	—
12 hours	0.65	2.54	—
24 hours	0.57	2.47	—
48 hours	0.57	2.24	—

[1] Temperature of water, 27°C.
[2] Autoclaved at 10 lb. pressure for 12 minutes.
[3] Temperature of water, 70°C.

and odor that render them unpalatable to those accustomed to milled, untreated (white) rice. These unattractive characteristics are due to fermentation and other changes that occur during the long period of preliminary soaking. In recent years, it has been found possible to greatly improve the quality of the product by soaking the padi in hot water (70°–90°C.) and curtailing the time of soaking to one or two hours (4, 5, 9).

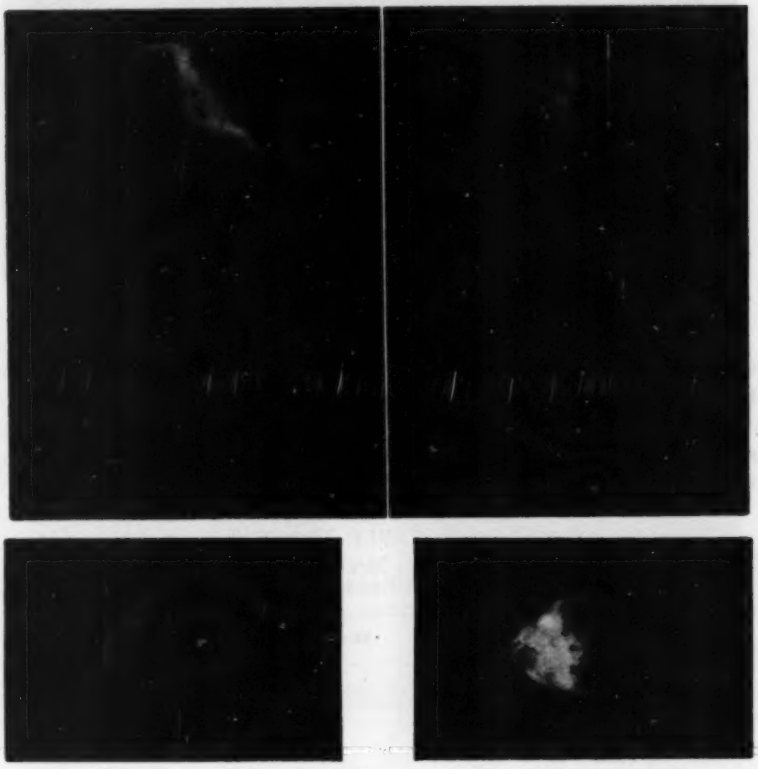


FIG. 4. Location of Riboflavin in hulled rice. *Top left.* Broad longitudinal section. *Top right.* Narrow longitudinal section. *Bottom left.* Cross section, center of grain. *Bottom right.* Cross section at germ of grain. Magnification: approx. 10 X.

In order to observe whether variations in the time of soaking and of the temperature of the soaking water cause any marked difference to the redistribution of thiamine in the grain, sample grains of hulled parboiled rice, derived from padi that had been subjected to different treatments, have been photographed. All sample batches were taken from a single bulk sample of locally grown padi. The thiamine

content of the hulled, untreated rice and of highly milled, untreated rice prepared from this padi was found to be as follows:

Hulled, untreated rice: 3.48 μg./g.
Milled, untreated rice: 0.60 μg./g.

The different treatments carried out and the thiamine content of the resulting highly milled products are shown in Table I. Thiamine was estimated by the method currently in use in our own laboratories

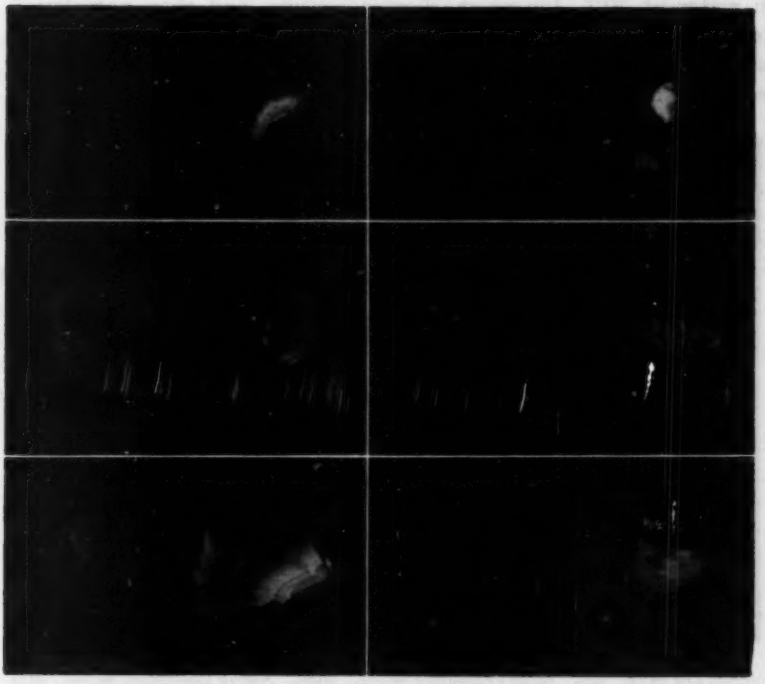


FIG. 5. Location of Thiamine in parboiled rice. *Top row.* No soaking. Autoclaved at 10 lb. pressure for 12 minutes. *Center row.* Soaked in cold water for 24 hours. Autoclaved at 10 lb. pressure for 12 minutes. *Bottom row.* Soaked in water at 70°C. for 2 hours. Autoclaved at 10 lb. pressure for 12 minutes. Magnification: approx. 7 ×.

(4). The rice was extracted with 0.1 *N* sulfuric acid, the extract incubated clarase, filtered, adsorbed on decalso and the thiamine eluted with potassium chloride solution. The thiamine in the eluate was oxidized to thiochrome with alkaline ferricyanide, extracted with iso-butanol and estimated using a Coleman photoelectric fluorimeter.

When photographs were examined of grains of hulled, parboiled rice, derived from padi that had been soaked in cold water (27°C.) without subsequent autoclaving, no indication could be found that any

thiamine had diffused into the endosperm, irrespective of the period of soaking. This is supported by the figures found for the thiamine content of these samples after they had been highly milled. Since no appreciable change from the untreated grains was visible, none of these photographs is reproduced here.

On the other hand, the samples of padi that had been soaked in cold water for different periods of time and subsequently autoclaved, appeared to correspond fairly closely with those that had been soaked in water at 70°C. for much shorter periods, and then autoclaved. In each case, the location of thiamine in the hulled rice derived from such samples of padi, as shown by photographs, was similar. For example, after autoclaving at 10 lb. pressure for twelve minutes,

FIG. 6. Location of Thiamine in commercial sample of highly milled, parboiled rice. Thiamine content: 2.03 μg./g. *Left.* Broad longitudinal section. *Right.* Narrow longitudinal section. Magnification: approx. 10 ×.

without prior soaking, a very slight diffusion of thiamine into the endosperm in the vicinity of the scutellum could be detected (Fig. 5 top row). This diffusion becomes more noticable as the time of soaking increases, both in cold and in hot water; and is clearly evident in the photographs of hulled rice derived from padi that had been soaked in cold water for 24 hours (Fig. 5 center row) or in water at 70°C. for two hours and subsequently autoclaved (Fig. 5 bottom row).

These photographs provide visual confirmation that a considerable proportion of the thiamine of the scutellum is absorbed into the endosperm during the parboiling process and would remain in the grain, even after it had been highly milled. This is supported by the

figures obtained for the thiamine content of the highly milled rice derived from these samples. (See Table I.) There are also clear indications that thiamine also diffuses into the endosperm from the pericarp and aleurone layers. Examination of these photographs has also shown that the concentration of thiamine in the scutellum has been considerably reduced. It would appear, therefore, that the main reason for the improved thiamine content of highly milled, parboiled rice as compared with highly milled, untreated rice is the diffusion of the thiamine into the endosperm from the scutellum and, to a smaller extent, from the pericarp layers. Even though the scutellum is rendered more adherent and so remains attached to the endosperm after milling, it appears that it would not contribute greatly to the thiamine content of the milled, parboiled rice, since it has already yielded much of its thiamine to the endosperm.

Thiamine in Commercial Samples of Parboiled Rice. Photographs have also been taken of grains from three samples of highly milled, commercial parboiled rice that had been purchased from local shops. The thiamine content of these samples was found to be: 1.24, 2.03, and 2.14 μg./g. respectively. Photographs of the sample with thiamine content of 2.03 μg./g. are reproduced in Fig. 6, and show that, although the germ and the greater part of the scutellum appear to have been removed, appreciable amounts of thiamine had diffused into, and had been retained by, the endosperm.

This work has only dealt with a comparatively small number of grains and with only a few of the many possible different parboiling treatments; and, so far, it has not been possible to obtain very clear results for riboflavin. Nevertheless, as Somers and his colleagues remark, the technique is so simple and convenient that it should prove most useful in further studies on rice and other cereals.

Acknowledgments

The author acknowledges, with thanks, the technical advice afforded him by Messrs. Kodak Limited, of Kingsway, London, in the selection of appropriate Wratten filters. He also wishes to record his grateful thanks to Mr. W. H. Ayton of the Survey Department, Federation of Malaya, for having made the screen and filter holder for attachment to the ultraviolet lamp.

Literature Cited

1. Acton, H. W., and Chopra, R. N. The problem of epidemic dropsy and beriberi. Ind. Med. Gaz. **60**: 1–18 (1925).
2. Altson, R. A., and Simpson, I. A. A note on the distribution of vitamin B_1 (thiamin) in the rice grain. Mal. Agric. J. **29**: 127–128 (1941).
3. Aykroyd, W. R. The effects of parboiling and milling on the antineuritic vitamin (B_1) and phosphate content of rice. J. of Hyg. **32**: 184–192 (1932).
4. Byron, F. E. Annual Report, Inst. Med. Research, Federation of Malaya; p. 20 (1949).
5. Done, J. The effects of various treatments of paddy rice on the aneurin and nicotinic acid content of the milled grain. Brit. J. Nutrit. **3**: 335–345 (1949).

6. GLOSTER, T. H. The aerobic spore-forming bacilli in rice. Indian Med. Research Mem. No. 10: 89–117 (1928).
7. HINTON, J. J. C. The distribution of vitamin B_1 in the rice grain. Brit. J. Nutrit. 2: 237–241 (1948).
8. HINTON, J. J. C. Parboiling treatment of rice. Nature 162: 913–915 (1948).
9. KIK, M. C. Private communication (1946).
10. NICHOLLS, L. Thiamine of milled raw and parboiled rice. Nature 160: 298 (1947).
11. SCHEUNERT, A., and SCHIEBLICH, M. Bildung von Vitamin B durch Bac. vulgatus (Flügge) Migula aus vitaminfreien Nährlösungen. Biochem. Z. 184: 58–66 (1927).
12. SCHEUNERT, A., and SCHIEBLICH, M. Über die Bildung von Vitaminen durch Bac. vulgatus und den Einfluss des pH der Nährlösung auf die Menge des gebildeten Vitamins B.
13. SIMPSON, I. A. Studies in vitamin B_1 (The antineuritic vitamin). Part II. The "Oryzatoxin" theory. Bull. Inst. Med. Research Federated Malay States (1934), No. 1, 24 pp.
14. SOMERS, G. F., COOLIDGE, M. H., and HAMNER, K. C. The distribution of thiamine and riboflavin in wheat grains. Cereal Chem. 22: 333–340 (1945).
15. SUBRAHMANYAN, V., SREENIVASAN, A., and DAS GUPTA, H. P. Studies on quality in rice. 1. Effect of milling on the chemical composition and commercial qualities of raw and parboiled rices. Ind. J. Agr. Sc. 8: 459–486 (1938).

POLYSACCHARIDES OF HIGH-AMYLOSE CORN [1]

WILLIAM DVONCH,[2] H. H. KRAMER,[3] AND ROY L. WHISTLER

ABSTRACT

Twelve true breeding endosperm types of corn involving the four gene loci *Su su^{am} su*, *Su$_2$ su$_2$*, *Du du*, and *Wx wx*, were analyzed for water-soluble polysaccharides, starch, and amylose content of the starch. With the exception of types involving *wx*, there was a negative correlation ($r = -0.81$) between amylose content and starch content. Alone, and in all combinations with other genes, *su* increases water-soluble polysaccharides to over 30%. The only other gene combinations which produced endosperms with more than 5% water-soluble polysaccharides were the *su^{am} du* and *su^{am} su$_2$ du* combinations.

Variability among non-isogenic lines within the genotypes studied suggests that modifying genetic factors as well as environmental factors are operating to modify carbohydrate interrelationships.

Starch granules of the various genotypes were different in microscopic appearance from normal corn starch and some gave other x-ray diffraction patterns. The starches with a high amylose content did not gelatinize in the usual fashion.

The amylose and amylopectin components of the starches were similar to the normal components except that the *su du* and *su^{am} su$_2$ du* amylopectins had end-group assays of 11 and 13, respectively as compared to assays of 20 to 30 for "normal" starch amylopectin, and low values of the intrinsic viscosities were found in some instances.

Because of the potential industrial value of amylose, much work has been done in an attempt to obtain this polysaccharide in com-

[1] Manuscript received September 25, 1950. Paper No. 472. Purdue University Agricultural Experiment Station, Department of Agricultural Chemistry.
[2] Present address: Northern Regional Research Laboratory, Peoria, Illinois.
[3] Department of Agronomy, Purdue University.

mercial quantities. One possible solution is the production of a corn genotype whose starch is largely, if not entirely, amylose. The effect of certain genes on the carbohydrate interrelationships in corn endosperm has been recently investigated by Cameron (6) and by Kramer and Whistler (10). In these investigations, it was found that the amylose content was raised from the value of approximately 25% of total starch for normal corn to 50 to 65% for a super-sugary type.

The present investigation is concerned with the amounts and natures of the water-soluble polysaccharides and starch, and of the amylose and amylopectin fractions present in the endosperm of various homozygous corn genotypes. These genotypes involve certain combinations of the four sets of alleles (*Su*, *su*^{am}, *su*), (*Su*$_x$, *su*$_x$), (*Du*, *du*), and (*Wx*, *wx*).[4] Aside from the extensive work on normal corn starch and its fractions and on waxy corn starch, only the water-soluble polysaccharides found in sugary endosperm have been investigated previously (7).

Materials and Methods

Production of the Genetic Material. Of the 29 samples shown in Table I, 22 were from genotypes grown and identified by self-pollination and test-crossing in 1949, two were from samples grown in 1948, four are from data of Cameron (6) and given here for comparison, and one is a commercial sample of Golden Bantam sweet corn. The normal starchy line used is a white endosperm "equivalent" of Inbred M14. Since the breeding procedure and methods of identifying the true breeding genotypes will be published elsewhere, it will not be described here. In four cases genotypic identification was not completely certain and these samples are so indicated in Table I. Where the genotype was established with certainty from the breeding methods employed, ears of the same genotype were bulked for analysis. In cases of doubt, individual ears were analyzed. In some cases it was not possible to isolate enough ears of the same genotype to give sufficient starch for fractionation.

Ears were dried immediately after harvest to 12–15% moisture with forced air at approximately 38°C. and stored in air-dry condition until used.

Isolation of Starch. Granular endosperm starch was isolated by a modified version of the method of Brimhall *et al.* (3). 50 g. of corn were soaked in distilled water for 10–30 minutes, after which the pericarp was peeled off, and the germ removed with a knife. In a few instances where difficulty was encountered in removing the pericarp, it was left on. The endosperm tissue was air-dried overnight and ground in a small Wiley mill to pass a 40-mesh sieve. The ground

[4] *su*^{am}, amylaceous sugary; *su*, sugary; *su*$_x$, sugary-x; *du*, dull; *wx*, waxy.

material was placed in a 250 ml. centrifuge bottle and extracted twice with 75% ethanol for 2-hour periods. In these, as well as other extractions, the mixture was continuously stirred. All extractions were performed at 25°. The extracted material was removed by centrifugation and was extracted overnight with a 0.2% water solution of sulfur dioxide. The sulfur dioxide solution was replaced with water, and the mixture agitated in a Waring blendor for a few minutes. The slurry was passed through coarse bolting cloth, and the protein and fiber again agitated in fresh water to wash out the remaining starch. The slurry was again filtered, and material not passing the bolting cloth was discarded. The starch and protein mixture was extracted with 0.06 *N* sodium hydroxide solution for 2 hours. The centrifuged precipitate was washed with water, neutralized to the phenolphthalein end-point with dilute hydrochloric acid, and centrifuged. The glutinous layer which formed was scraped off, and the starch resuspended and recentrifuged until a glutinous layer no longer formed. The starch was suspended in fresh water and passed through a No. 25 standard silk bolting cloth to remove any remaining fiber. The clean starch was extracted for two days with 85% methanol in a Soxhlet apparatus, removed from the thimble, air-dried, and ground to pass a 60-mesh screen.

In a few cases where it was difficult to obtain a starch of satisfactory purity, the starch was given additional extractions with 75% ethanol and 0.06 *N* sodium hydroxide solutions. Sometimes it was helpful to isolate the glutinous layer while the mixture was alkaline.

The yields of starch obtained varied widely, ranging from 4–61%, based on endosperm tissue, or 3–51%, based on the whole corn. The recovery of starch varied from about 26 to about 100%. This latter variation reflects the difficulty in obtaining starch from a few of the samples.

Micro-Kjeldahl determinations on the starches indicated protein contents which varied from 0.23–0.93%. As a correction for protein content would not significantly alter any of the other reported results, it was ignored.

Starch Fractionation Procedure. As only small quantities of starch were available, the procedure of Lansky *et al.* (11) was modified to make the initial starch concentration 1% for the Pentasol fractionation. The precipitated amylose was recrystallized twice with *n*-butanol from solutions of approximately the same volume.

Analytical Methods. To obtain data comparable to those of Cameron (6), his method of analysis for water-soluble polysaccharides and starch was used.

Moistures were determined on all samples by drying *in vacuo* at 100° overnight, and all data are reported on a dry-weight basis.

Iodine sorptive capacities of the starches were obtained by the usual potentiometric titration (1, 13). Iodine sorbed was calculated from the inflection point, and amylose content of the starches was calculated by assuming that "pure" amylose sorbs 200 mg. iodine per gm. Experiments showed that the iodine sorption values were reproducible to ± 1% for the starches and ± 0.3% for the amyloses.

All x-ray diffraction patterns were obtained by exposing for 2 to 3 hours a 1 mm. thick sample of the wet starch to nickel filtered copper radiations at 35 K.V.P., 23 ma., and a specimen-to-film distance of 5.0 cm. in a flat film camera. The sample was contained between Scotch tape which did not interfere with the identification of the A and B starch patterns. Intrinsic viscosities were determined from solutions in 1 *N* sodium hydroxide solution. Amylopectin was dispersed by the procedure of Lansky *et al.* (11).

Periodate end-group assays were done by the method of Hirst *et al.* (4, 8).

Cupric chloride crystallization patterns were made by the procedure of Morris and Morris (12).

Results and Discussion

Gene Effects. The various lines within specific genotypes shown in Table I were not isogenic with respect to their genetic backgrounds. The variation within genotypes suggests, therefore, either that there may be modifying genetic factors, not yet identified, which affect the carbohydrate interrelationships or that environmental effects may cause variation. That both are operating seems probable. The variability within genotypes is further emphasized when lines grown in 1948, Cameron's lines, and Golden Bantam sweet corn are included.

The effects of individual genes and gene combination, however, are pronounced in spite of the variability within genotypes. Figures 1 and 2 relate amylose content of the starch to the amount of starch and to the per cent of water-soluble polysaccharides. For these comparisons, averages for each genotype in Table I were used excluding samples of uncertain genotype.

With the exception of the *wx* gene effects, Fig. 1 shows a general inverse relationship between per cent amylose in the starch and starch content of the endosperm irrespective of the genotypes involved. The correlation coefficient for the 11 comparisons was found to be − 0.81, a highly significant value.

The relationship between amylose content and per cent water-soluble polysaccharides, on the other hand, is much more clearly dependent on specific genotypes (Fig. 2). The effect of the *su* gene is most striking. Alone, or in combination with *wx*, su_s, or *du*, it increased the water-soluble polysaccharides to more than 30% and

TABLE I

GENETIC CONSTITUTION, ENDOSPERM DESCRIPTION, AND QUANTITIES OF POLYSACCHARIDES IN 13 HOMOZYGOUS CORN ENDOSPERM TYPES

Sample	Homozygous Recessive Genes Present	Endosperm Description	Water-soluble Polysaccharides in Endosperm	Starch in Endosperm	Amylose in Starch		Portion Not Soluble After Autoclaving 3 Hours at 124°
					Based on 200 mg. I_2/g. Amylose	Based on Determined I_2 Sorption	
			%	%	%	%	%
1	None	Normal	0.1	74	25		
2	*du*	Dull	0.2	67	34	37	2
3	su_s	Sugary-x	0.2	46	42		
4	su_s	Sugary-x	0.3	60	37	38	7
5[2]	su_sdu	Sugary-x dull	0.1	61	39		
6[2]	su_sdu	Sugary-x dull	Trace	63	39	42	4
7	su_sdu	Sugary-x dull	0.4	45	52		
8[1]	su^{am}	Pseudostarchy	0.4	81	31		
9[1]	$su^{am}du$	Amylaceous sugary	14	52	50		
10	$su^{am}du$	Amylaceous sugary	8	42	44		
11	$su^{am}du$	Amylaceous sugary	12	46	42		
12	$su^{am}du$	Amylaceous sugary	2	32	44	50	7
13	$su^{am}du$	Amylaceous sugary	4	38	43		
14	$su^{am}du$	Amylaceous sugary	7	48	42	49	13
15	$su^{am}su_sdu$	—	12	34	53	58	24
16[1]	*su*	Sugary	33	32	39		
17	*su*	Sugary	27	42	30	32	
18	*su*	Sugary	44	17	34		
19[2]	*su*	Sugary	18	41	31	34	2
20[1]	*sudu*	Supersugary	41	19	65		
21	*sudu*	Supersugary	28	14	64		
22	*sudu*	Supersugary	33	12	60	66[3]	31[3]
23	*sudu*	Supersugary	38	16	56		
24	$susu_s$	Supersugary-x	42	13	40		
25	$susu_s$	Supersugary-x	38	14	54		
26	$susu_sdu$	—	34	10	63		
27[2]	$susu_sdu$	—	41	11	55		
28	*wx*	Waxy	4	72	0		
29	*suwx*	Sugary waxy	32	27	0		

[1] Data of Cameron (6).
[2] Genetic classification not certain.
[3] Values for 3 samples bulked, see Table III.

reduced the starch content to less than 30%. With respect to amylose content, it is masked by *wx;* it interacts strongly with *du* to increase amylose content, as shown by Cameron and confirmed here, and appears to act independently of su_s as shown by the fact that the differences in amylose content between the normal and su_s genotypes

and between the *su* and *su su$_z$* genotypes are very similar. Alone, it appears to increase amylose only slightly.

The action of the genes *su$_z$* and *du* is similar in that they appear to increase the amylose content and decrease the starch content without increasing the water-soluble fraction appreciably. The two act independently since the increase in amylose content in the *su$_z$ du* endosperm is approximately the sum of the increases shown by *su$_z$* and *du* endosperms separately; however, *du* interacts with *su* while *su$_z$* does not.

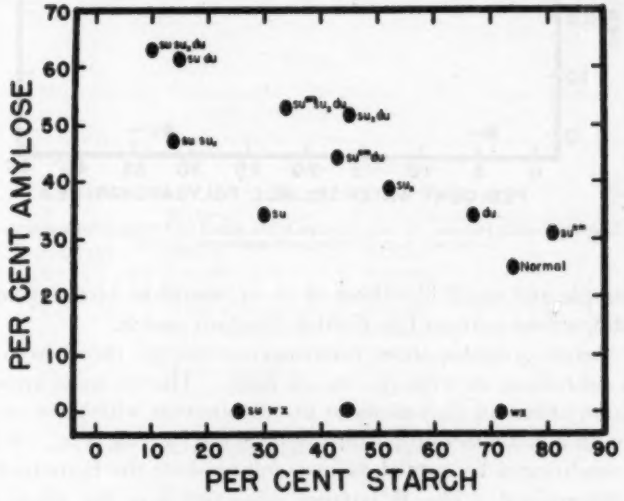


FIG. 1. The relationship between per cent amylose in the starch and starch content of the endosperm of 13 homozygous genotypes in corn.

The triple recessive *su su$_z$ du* was not greatly different from the double recessive *su du* but the *su^{em} su$_z$ du* genotype had more amylose, less starch, and more water-soluble polysaccharides than did the *su^{em} du* type.

Characteristics of the Starch Granules. Starch from each of the various genotypes available was examined microscopically and by x-ray diffraction. The data are summarized in Table II, which also contains data on normal and waxy starch for comparison.

There is a relationship between microscopic appearance and x-ray diffraction pattern and the genotypes investigated here although in some cases there are apparent exceptions. The reason for the doubtful visual classification of sample 49-24 as an *su* type becomes clear when it is found that the granules differ from those of Golden Bantam in

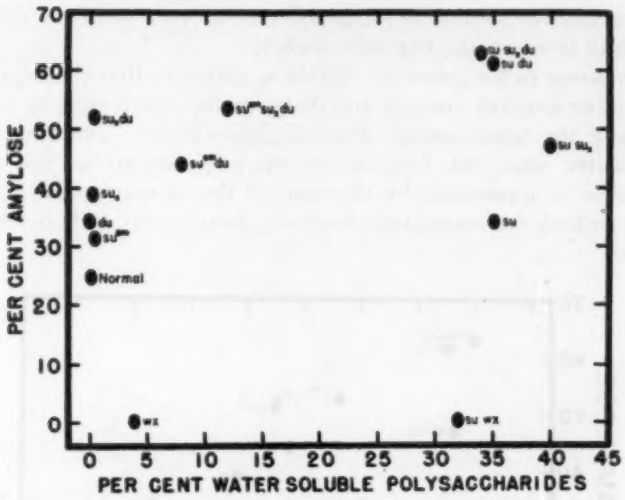


FIG. 2. The relationship between per cent amylose in the starch and water-soluble polysaccharides in the starch in 13 endosperm genotypes of corn.

being simple and small like those of *su* su_z yet show birefringence and the A diffraction pattern like Golden Bantam starch.

All starch granules show birefringence except those from endosperms containing *su* with su_z, *du*, or both. The su_z gene appears to differ from others in that alone or in combination with *du* it produces starch with a B diffraction pattern as does the su^{am} *du* type. Whether starch conditioned by su^{am} alone also will produce the B pattern needs to be determined. The B pattern observed here for some of the

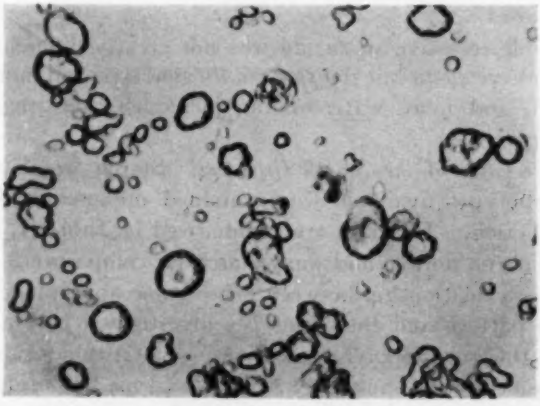


FIG. 3. Starch granules from No. 35 (supersugary) starch.

starches was once thought to be characteristic of potato starch, but has been similarly found in a pea starch of high amylose content by Hilbert and MacMasters (9). In some of the B patterns, the line corresponding to the interplanar spacing of 15.8 Å (2) was much weaker than in a typical pattern. It must be realized, however, that there exists a graded series of patterns between the A and B types and great emphasis should not be placed on the small variations found here. Like this pea starch, the various high-amylose corn starches were incompletely gelatinized by autoclaving (3 hours at 124°C.). The amount of undissolved material parallels amylose content (Table I).

TABLE II

MICROSCOPIC APPEARANCE AND X-RAY DIFFRACTION DATA FOR VARIOUS STARCHES

Sample Number	Genotype	Microscopic Appearance			X-Ray Diffraction Pattern
		Simple or Compound	Size and Shape	Birefringence	
49-37	Normal	Simple	Normal	+	A
49-36	*wx*	Simple	Normal	+	A
49-38	*suwx*	Simple	Small	+	A
49-18	*du*	Many compound	Normal	+	A
G.B.	*su*	Many compound	Normal	+	A
49-24	*su*	Simple	Small	+	A
49-19	su_z	Mostly compound	Normal	+	B
49-21	$su_z du$	Many compound	Normal	+	B
49-9	$su^{am} du$	Many compound	Normal	+	B
48-13	$su^{am} du$	Many compound	Normal	+	B
49-30	$su^{am} su_z du$	Many compound	Normal	+	B
49-27	$susu_z$	Simple	Small	−	C[1]
48-28	$susu_z$	Simple	Small	−	C[1]
49-35	*sudu*	Mostly simple	Misshapen	−	D[2]
49-29	$susu_z du$	Simple	Small	−	None

[1] This pattern contains 4 lines of the B pattern (2) with different intensities. *d*, Å: 15.5, very faint; 5.9, very faint; 5.2, faint; 4.5, moderate. The last line might be due to the Scotch tape holder.
[2] *d*, Å: 6.2, very faint; 5.4, faint; 4.6, faint. The last line might be due to the Scotch tape holder.

From the above, it is evident that the use of gelatinization temperature as a method of characterization is rather meaningless for these starches. Furthermore, loss of anisotropy which is used as one criterion of gelatinization does not occur simultaneously with appreciable swelling and breaking up of granule structure. For example, starch from sample 48-13, containing 42% amylose, lost anisotropy at 65–70°C. which is the gelatinization range of normal starch, but most granules were not swollen. At 80°C. about half the granules had the characteristic gelatinized appearance, but after heating for 2 hours at 120°C., 13% could be recovered by centrifugation. This insoluble material may be swollen but undisintegrated granules. It had an

iodine sorption capacity identical with the original starch, indicating that no fractionation had occurred.

Characteristics of the Amylose and Amylopectin. The iodine sorption values for the amyloses are variable (Table III), as they are for normal corn starch amyloses. Some values are lower than those for normal amylose, but there is no readily apparent correlation with gene action.

Lansky *et al.* (11) have shown that iodine sorption is different for amyloses from different starches and that it is not correct to assume that "pure" amylose absorbs 200 mg. of iodine per g. The amylose content of a starch should be calculated from the maximum iodine

TABLE III

PROPERTIES OF THE AMYLOSE AND AMYLOPECTIN ISOLATED FROM VARIOUS STARCHES

No.	Starch Type	Amylose		Amylopectin	
		Iodine Sorption	Intrinsic Viscosity	End-Group Assay	Intrinsic Viscosity
		mg./g.			
49-37	Normal	188.7	1.04	21	1.15
49-36	*wx*	—	—	19	—
49-38	*suwx*	—	—	19	—
49-18	*du*	184.2	0.90	21	0.90
G. B.	*su*	187.5	1.33	18	—
49-24	*su*	182.9	0.72	20	1.02
49-24	su_z	194.3	0.77	20	1.15
49-21	su_zdu	186.4	0.69	19	0.97
49-32	$su^{am}du$	177.0	0.58	18	0.35
48-13[1]	$su^{am}du$	172.0	1.08	17	
49-30	$su^{am}su_zdu$	181.9	0.68	13	
49-25, 26, 35[2]	$susu_z$	175.8	0.75	11	

[1] Recrystallized once.
[2] Bulked sample, 6 per cent No. 25, 15 per cent No. 26, 79 per cent No. 35.

sorption of its amylose. When this is done for the starches listed in Table III, the results shown in column 7 of Table I are obtained. These values are appreciably higher than those calculated on the assumption of an iodine sorption of 200 mg./g. and are more nearly truly representative of amylose content. For routine analysis of a large number of starch samples, some arbitrary value for amylose sorption must be taken. A value of 190 mg./g., which is the average value found for a number of normal corn amyloses, would seem appropriate.

It has been suggested that a substance intermediate between amylose and amylopectin occurs in corn starch, to the extent of 5–7% of the starch (11). This material is precipitated by Pentasol, but not

by *n*-butanol. The filtrates from the first *n*-butanol recrystallization in the case of No. 30 and No. 25, 26, 35 amylose on treatment with 2 volumes of methanol yielded an insignificant amount of precipitate. This intermediate material was probably not present in greater amount in starch No. 30 and 25, 26, 35 than in normal starch.

Dvonch and Whistler (7), in discussing the nature of the water-soluble polysaccharides of sweet corn, suggested that amylopectins with various degrees of branching will be found. The amylopectins of the *su du* and *su*^{am} *su*$_x$ *du* genotypes with periodate end-group assays of 11 and 13 are the first examples of amylopectins intermediate in degree of branching between glycogen and amylopectins of normal starch. The cupric chloride crystal pattern of the *su*^{am} *su*$_x$ *du* amylopectin was also intermediate in appearance between the amylopectin and water-soluble polysaccharide pattern. This further supports the view that water-soluble polysaccharides of corn should be considered amylopectins. Since normal end-group assays of 20 to 30 (5) were obtained for the other amylopectins, there is as yet no readily apparent genetic hypothesis for the formation of these two highly branched amylopectins.

The number of genotypes analyzed for intrinsic viscosities of the amylose and amylopectin fraction is too small to establish the relation with genetic constitution. While the values are lower than those given by Lansky *et al.* (11) for normal corn starch fractions, it is possible that similar values would be found in starches not chosen for the highest "hot paste viscosity."

Acknowledgments

The authors wish to thank Mr. Gerald Dunn of the Department of Agronomy for the production of the genetic material, Dr. H. H. Yearian of the Department of Physics for obtaining the x-ray diffraction patterns, Mr. L. C. Shenberger and Mr. Lee House for the photomicrographs, and Elsie Mihelich Armstrong for much of the analytical work.

Literature Cited

1. Bates, F. L., French, D., and Rundle, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65**: 142–148 (1943).
2. Bear, R. S., and French, D. The significance of x-ray diffraction patterns obtained from starch granules. J. Am. Chem. Soc. **63**: 2298–2305 (1941).
3. Brimhall, B., Sprague, G. F., and Sass, J. E. A new waxy allel in corn and its effect on the properties of the endosperm starch. J. Am. Soc. Agron. **37**: 937–944 (1945).
4. Brown, F., Dunstan, Sonia, Halsall, T. G., Hirst, E. L., and Jones, J. K. N. Application of new methods of end-group determination to structural problems in the polysaccharides. Nature **156**: 785–786 (1945).
5. Brown, F., Halsall, T. G., Hirst, E. L., and Jones, J. K. N. The structure of starch. The ratio of nonterminal to terminal groups. J. Chem. Soc. 27–32 (1948).
6. Cameron, J. W. Chemico-genetic bases for the reserve carbohydrates in maize endosperm. Genetics **32**: 459–485 (1947).

7. DVONCH, W., and WHISTLER, R. L. Water-soluble polysaccharides of sweet corn. J. Biol. Chem. **181**: 889–895 (1949).
8. HALSALL, T. G., HIRST, E. L., and JONES, J. K. N. The structure of glycogen. Ratio of nonterminal to terminal glucose residues. J. Chem. Soc. 1399–1400 (1947).
9. HILBERT, G. E., and MACMASTERS, M. M. Pea starch, a starch of high amylose content. J. Biol. Chem. **162**: 229–238 (1946).
10. KRAMER, H. H., and WHISTLER, R. L. Quantitative effects of certain genes on the amylose content of corn endosperm starch. Agron. J. **41**: 409–411 (1949).
11. LANSKY, SYLVIA, KOOI, MARY, and SCHOCH, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. **71**: 4066–4075 (1949).
12. MORRIS, D. L., and MORRIS, CAROL T. Special effects of certain tissue extracts on the crystallization patterns of cupric chloride. J. Phys. Chem. **43**: 623–629 (1939).
13. WILSON, E. J., JR., SCHOCH, T. J., and HUDSON, C. S. The action of *macerans* amylase on the fractions from starch. J. Am. Chem. Soc. **65**: 1380–1383 (1943).

UTILIZATION OF FUNGAL AMYLASE FOR ALCOHOL PRODUCTION [1,2]

JULIAN CORMAN and H. M. TSUCHIYA

ABSTRACT

The present study confirms previous findings that the alcohol yield from grain mashes converted with fungal amylase is positively correlated with the maltase content of the latter. However, this correlation exists only if an adequate amount of alpha-amylase is also present.

The supplementation of fungal amylase containing moderate amounts of alpha-amylase and maltase with small amounts of barley malt does not spare the amount of fungal amylase required for satisfactory conversion of grain mashes. However, the supplementation of fungal amylase high in maltase but low in alpha-amylase, with small amounts of malt, lowers substantially the requirement of fungal amylase.

Conversion of grain mashes at temperatures as high as 65°C. is more advantageous than at lower temperatures because more rapid liquefaction is effected. Fungal maltase is more thermostable than fungal alpha-amylase. This is in contrast to the amylolytic enzymes of barley malt where the saccharifying enzyme is less thermostable than the alpha-amylase.

The fungal amylase process has been found adaptable to the production of alcohol from farinaceous materials other than corn.

The replacement of distillers' malt with culture liquors of certain strains of *Aspergillus niger* grown in aerated submerged cultures was first reported by Van Lanen and LeMense (10) in 1946. Pilot-plant investigations (5) indicated that appreciable savings could be realized in distillery operation by replacing malt with fungal amylase. Since the initial reports (4, 10), a number of papers have appeared pertaining

[1] Manuscript received October 13, 1950. Presented at the Annual Meeting, May, 1949.
[2] Contribution from the Fermentation Division, Northern Regional Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

to the optimal conditions for producing mold enzymes (1, 3, 9). However, reports on the utilization of fungal amylase in the production of alcohol from grain have been lacking. Hence, the present study was undertaken.

Materials and Methods

Alpha-amylase was determined by the procedure of Sandstedt, Kneen and Blish (7) as modified by Olson, Evans, and Dickson (6). A unit of alpha-amylase is defined as that amount of enzyme which dextrinizes 1 g. of starch, pretreated with excess beta-amylase, at 20°C. in 1 hour.

Maltase activity was determined by measuring the extent of hydrolysis of maltose monohydrate to glucose as indicated by the increase of reducing power of a reaction mixture of maltose and culture filtrate (9). A unit of maltase is defined as that amount of enzyme which hydrolyzes 1 mg. of maltose monohydrate to glucose in 1 hour at 30°C. This unit is equivalent to approximately 10 units used in our previous studies (2, 4, 5).

Alcohol yield was determined as follows: A slurry of 50 g., or 50 g. less the amount of barley malt, of corn meal (12% moisture) in 200 ml. of tap water contained in a 500-ml. Erlenmeyer flask was "cooked" at 130°C. for 30 minutes and then cooled to approximately 75°C. A 50-ml. portion of mold culture filtrate or slurry of barley malt and tap water, which further cooled the mash, was added with vigorous stirring. The mixture of grain mash and converting agent was then held at 60°C. for 10 minutes in a water bath. The converted mash was subsequently cooled to 30°C. and inoculated with 8 ml., representing an inoculum of 3.2%, of a 24-hour culture of *Saccharomyces cervisiae* NRRL Y-567. After fermentation at 30°C. for 72 hours the whole beer was transfered quantitatively to an 800-ml. Kjeldahl flask and distilled. Ninety-eight to 99 ml. of distillate was collected into a 100-ml. volumetric flask and made up to volume. The alcohol content was determined by a refractometric method.

In all cases, the alcohol yield was corrected for the alcohol introduced by the yeast inoculum. In addition, the yields from mashes converted with fungal amylase were corrected for the corn in the medium used to propagate the mold. Thus, the yields from the mashes converted by mold amylase are computed in terms of the total grain used, namely, the 50 g. of corn in the slurry plus the corn used in the medium for propagating the mold. Undoubtedly, a portion of the starch in the grain used in the fungal amylase production medium was metabolized by the mold and, therefore, the total potential starch was unavailable to the yeast for fermentation to alcohol. Therefore, this

method of calculation gives the actual expected yield of alcohol per bushel of grain processed in a plant using fungal amylase. To produce the fungal amylase preparations used in the investigation, selected molds were grown in 1-l. Erlenmeyer flasks containing 200 ml. of corn meal and distillers' thin stillage, adjusted after sterilization to pH 5.1 with sodium hydroxide. Cultures were aerated at 30°C. for 6 days on a reciprocating shaker making 95 complete 3-inch strokes per minute. Although distillery plant practice would call for the utilization of the entire mold culture in the conversion process, it was more convenient to filter off the mold and use the filtrate in the laboratory experiments.

Results and Discussion

The Effect of Maltase and Alpha-Amylase on Production of Fermentable Sugars. The significant role played in the production of fermentable sugars from starch by a carbohydrase enzyme present in mold culture liquors, other than alpha-amylase, was demonstrated by Schwimmer (8) and confirmed by Corman and Langlykke (2). Inasmuch as activity can be measured by the hydrolysis of maltose, the enzyme has been referred to as "maltase." Again, evidence suggesting that this enzyme also produces glucose directly from polymers of glucose higher than maltose has resulted in its being termed also the "glucogenic enzyme." It was previously found that alcohol yields from corn mashes converted with culture filtrates of 15 different species of *Aspergillus* and *Rhizopus* correlated with the maltase contents (2).

Recently (9), it was found possible to control the maltase activity of cultures of *Aspergillus niger* NRRL 337 by altering the medium composition. To ascertain the existence of a correlation between alcohol yields and maltase contents of cultures of a single strain of mold, corn mashes saccharified with culture filtrates of varying maltase level at the rate of 2.7 gal. per bu. of corn were fermented to alcohol. The data in Fig. 1 confirm our previous finding of a positive correlation between alcohol yields and the amounts of maltase in fungal amylase. Moreover, the data also illustrate the definite necessity of low but adequate amounts of alpha-amylase for satisfactory alcohol yields. Alcohol yields lower than those predicted by measurement of maltase activity alone were obtained when the alpha-amylase potency was less than 1 unit per ml. Since both enzymes are required for satisfactory alcohol yields, the amounts of maltase and alpha-amylase in mold culture filtrates have been used to characterize the fungal amylase preparations in this study.

Supplementation of Fungal Amylase with Malt. Since it might be desirable in some situations to supplement the action of fungal amylase with malt, the alcohol yields from corn mash saccharified with com-

binations of mold culture filtrates and malt were also investigated. The molds were grown in media containing 5% distillers' thin stillage solids and 5% corn meal. The *Aspergillus niger* NRRL 337 culture filtrate used contained 9.3 maltase units and 15.0 alpha-amylase units per ml. The malt used in these experiments was a 196° Lintner malt assaying 39 alpha-amylase units per gram.

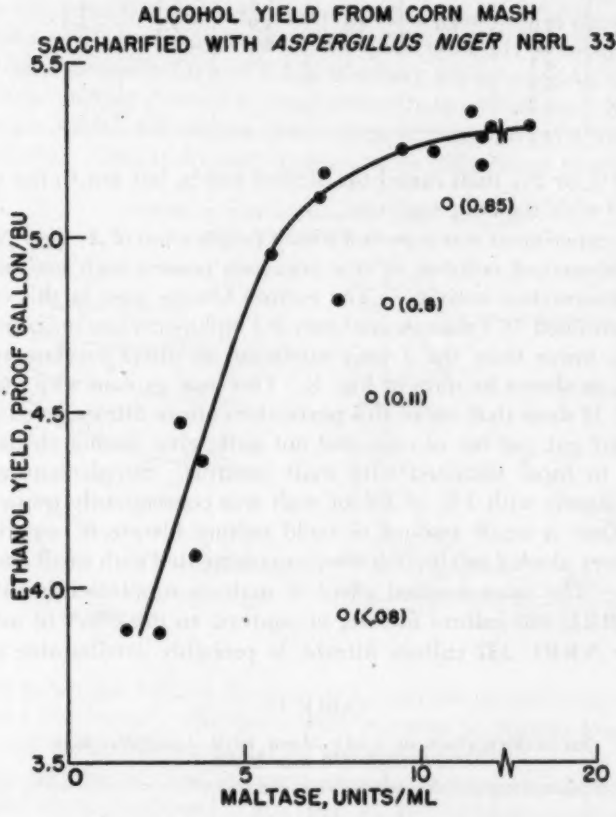


FIG. 1

The data in Table I show that this particular culture filtrate gave an alcohol yield comparable to those of malt controls when used at the rate of 1.7 gal. per bu. of corn for saccharification purposes. This represents utilization of mold culture for conversion purposes at the rate of 5% by mash volume where a 36-gal. beer is fermented. When this culture filtrate is used at rates less than 1.7 gal. per bu., the addi-

TABLE I

SACCHARIFICATION OF CORN MASH WITH *Aspergillus niger* NRRL 337 AND MALT

Fungal Amylase, gallons per bushel	0	0.34	0.68	1.0	1.4	1.7	2.7
Malt, per cent	*Alcohol yield, proof-gallons per bushel*						
0	—	—	—	4.5	5.1	5.3	5.3
1	4.0	4.6	4.8	4.9	5.2	5.3	5.4
2	4.5	5.1	5.1	5.1	5.1	5.2	
3	4.7						
8	5.2						
10	5.2						

tion of 1% or 2% malt raised the alcohol yields, but not to the values obtained with the malt controls.

This experiment was repeated with a preparation of *A. niger* NRRL 330. Submerged cultures of this organism possess high maltase but low alpha-amylase activity. The culture filtrate used in this experiment contained 38.3 maltase and only 0.7 alpha-amylase units per ml., which is lower than the 1 unit minimum of alpha-amylase recommended, as shown by data in Fig. 1. This may explain why the data in Table II show that use of this particular culture filtrate, even at the rate of 2.7 gal. per bu. of corn, did not quite give alcohol yields comparable to those obtained with malt controls. Supplementing this culture filtrate with 1% or 2% of malt was consequently quite beneficial. Only a small amount of mold culture filtrate is required for satisfactory alcohol production when supplemented with small amounts of malt. The more marked effect of malt in supplementing the *A. niger* NRRL 330 culture filtrate, in contrast to the effect of malt on *A. niger* NRRL 337 culture filtrate, is probably attributable to the

TABLE II

SACCHARIFICATION OF CORN MASH WITH *Aspergillus niger* NRRL 330 AND MALT

Fungal Amylase, gallons per bushel	0	0.34	0.68	1.0	1.4	1.7	2.7
Malt, per cent	*Alcohol yield, proof-gallons per bushel*						
0	—	3.3	4.9	5.0	—	5.1	5.1
1	4.0	5.0	5.2	5.2	5.2	5.2	5.3
2	4.5	5.0	5.2	5.2	5.3	5.3	
3	4.7	5.1	5.2	5.3	5.3	5.3	
5	5.2						
8	5.2						

alpha-amylase of malt supplementing the high maltase but low alpha-amylase content of the *A. niger* NRRL 330 culture filtrate. If for any reason it becomes mandatory to use some malt as an adjunct to mold amylase in the conversion of grain mash, *A. niger* NRRL 330 may be the preferred organism over *A. niger* NRRL 337.

Heat Stability of Maltase and Alpha-Amylase. Fungal amylases are generally considered highly susceptible to thermal inactivation. However, it appeared desirable to measure the heat stability of the maltase and alpha-amylase of our preparations. Since the pH of cooked grain mash varies between 5.0 and 5.5 in distillery plant practice, the heat stability of mold enzymes was measured at both pH values. Samples of *A. niger* NRRL 337 culture filtrate were adjusted to pH 5.0 and 5.5, respectively. Twenty-five-ml. aliquots were transferred to 25 × 200

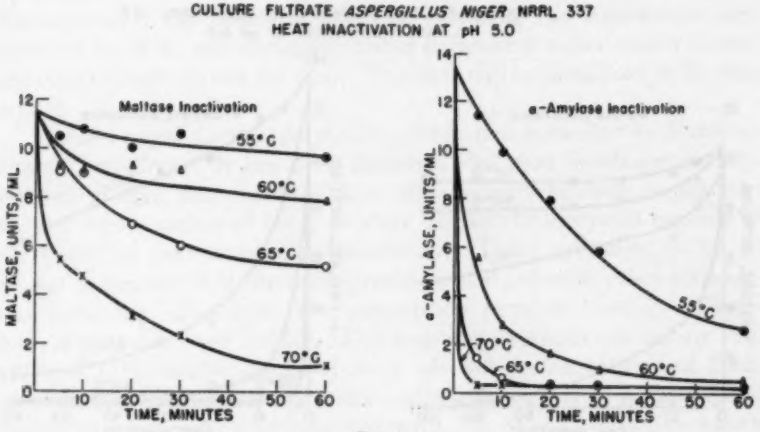


FIG. 2

mm. test tubes and held at the desired temperatures in constant temperature water baths. The tubes were removed at regular intervals, cooled, and the contents assayed for enzyme activities.

Alpha-amylase of mold is inactivated by heat more rapidly than the accompanying maltase. Thus (Fig. 2), 97% of alpha-amylase was inactivated in 20 minutes at 65°C. whereas only 45% of maltase was destroyed under identical conditions. Therefore, the situation is the reverse of that in barley malt where the saccharifying enzyme is less heat-stable than the dextrinogenic amylase. The mold was grown in medium lacking calcium and the presence of calcium ions might have increased the thermostability of the alpha-amylase.

Data from Figs. 2 and 3 show that alpha-amylase was somewhat less heat-stable at pH 5.0 than at 5.5. For example, at 60°C. 80% of

the alpha-amylase was destroyed in 10 minutes at pH 5.0, whereas only 55% was inactivated at pH 5.5.

On the other hand, maltase was somewhat more heat-stable at pH 5.0 than at 5.5. At 70°C., there was a 55% destruction of maltase in 10 minutes at pH 5.0, compared to 70% inactivation at pH 5.5.

Mash Conversion at Varying Temperatures and Times. During the saccharification of mash, the fungal amylolytic systems may be protected against heat inactivation by the starch substrate. Therefore, the heat-stability studies required extension to determinations of the optimal time and temperature for mash conversion with fungal amylase. The culture filtrate of *A. niger* NRRL 337 used in this experiment contained 8.6 maltase units and 14.2 alpha-amylase units per ml. The corn mashes, converted with culture filtrates at the rate of 2.7 gal. of

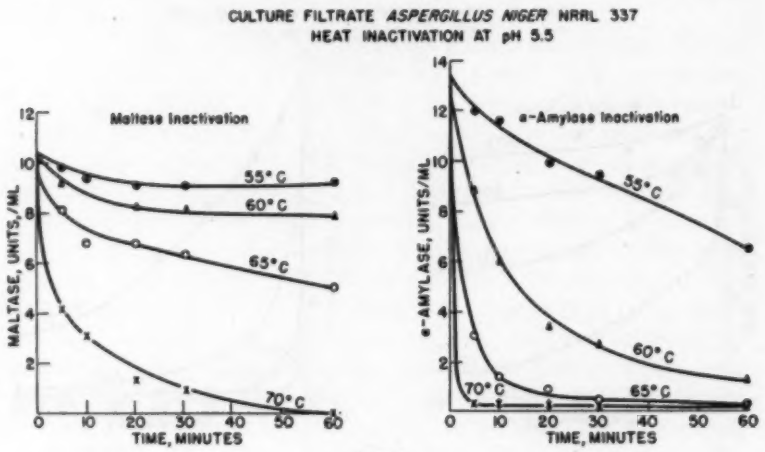


FIG. 3

fungal amylase liquor per bu. of corn, were held at 55°, 60°, 65°, and 70°C. for 5 to 30 minutes. At regular intervals the converted mashes were removed and cooled to 30°C. Subsequently, they were fermented with yeast.

Data from Table III show that satisfactory alcohol yields were obtained at conversion temperatures of 50° to 65°C., depending upon the holding times. It will be noted that satisfactory alcohol yields were obtained at 60° to 65°C. despite the inactivation of alpha-amylase at these temperatures as seen in the heat stability experiments. This may be attributed to the protective action of starch substrate on alpha-amylase as well as to the greatly accelerated cleavage of the starch before enzyme inactivation.

TABLE III

EFFECT OF CONVERSION TEMPERATURE ON ALCOHOL YIELD

Temperature, °C.	50	55	60	65	70
Time, minutes	*Alcohol yield, proof-gallons per bushel*				
5	5.1	5.3	5.3	5.1	4.6
10	5.1	5.2	5.3	5.3	4.5
20	5.2	5.2	5.3	4.8	4.6
30	5.2	5.2	5.2	4.4	4.5

Although yields at the higher temperatures were not higher than those obtained at 55°C., liquefaction of the corn mashes proceeded considerably faster at the higher temperatures. This is of significance where the converted mash must be pumped through pipe lines in plant operation. The more fluid mash, of course, makes for easier pumping. Subsequently, the beneficial effect of elevating the conversion temperature to 68°C. was demonstrated in plant-scale experiments involving 6000 bushels of corn per run. The data will be presented in another report.

Use of Fungal Amylase for Alcohol Production from Starchy Materials Other Than Corn. It has been demonstrated that molds grown submerged in corn and distillers' thin stillage, derived from a previous alcohol fermentation of corn, produce amylolytic enzymes capable of saccharifying corn mashes satisfactorily. There appeared to be no reason to suspect that the same process would fail with other farinaceous materials. However, the assumption required testing. Therefore, *Aspergillus niger* NRRL 337 was grown in media containing thin stillages (5% solids) obtained from alcohol fermentations of mold amylase converted mashes of wheat, milo sorghum, dried potatoes, and mold-damaged corn, supplemented with 5% of the respective starch materials. These mold culture filtrates were then used as converting

TABLE IV

ADAPTATION OF FUNGAL AMYLASE PROCESS TO VARIOUS FARINACEOUS MATERIALS

Sample	Enzyme Potency of Mold Cultures		Ethanol Yields, Proof-Gallons per 56 Lbs.	
	Maltase, units per ml.	Alpha-amylase, units per ml.	Mold Converted Mashes	Malt Converted Mashes
Corn	9.3	15.0	5.3	5.2
Moldy corn	8.2	9.6	4.8	4.8
Wheat	16.0	10.2	4.7	4.7
Milo sorghum	13.5	8.9	5.0	4.7
Dried potatoes	9.4	13.8	5.4	5.4

agents at the rate of 2.7 gal. per 56 lbs. of the corresponding farinaceous materials. Control mashes in which malt was used as converting agent at the level of 10% of the grain bill were fermented simultaneously in each instance. The data in Table IV show that *A. niger* NRRL 337 culture filtrates satisfactorily saccharified wheat, milo sorghum, dried potatoes, and moldy-corn mashes. The alcohol yields obtained from fungal amylase converted mashes equalled or exceeded slightly those from the malt converted controls.

Literature Cited

1. Adams, S. L., Balankura, B., Andreasen, A. A., and Stark, W. H. Submerged culture of fungal amylase. Ind. Eng. Chem. **39**: 1615–1617 (1947).
2. Corman, Julian and Langlykke, A. F. Action of mold enzymes in starch saccharification. Cereal Chem. **25**: 190–201 (1948).
3. Erb, N. M., Wisthoff, R. T., and Jacobs, W. L. Factors affecting the production of amylase by *Aspergillus niger* strain NRRL 337 when grown in submerged culture. J. Bact. **55**: 813–821 (1948).
4. LeMense, E. H., Corman, J., Van Lanen, J. M., and Langlykke, A. F. Production of mold amylases in submerged culture. J. Bact. **54**: 149–159 (1947).
5. LeMense, E. H., Sohns, V. E., Corman, J., Blom, R. H., Van Lanen, J. M., and Langlykke, A. F. Grain alcohol fermentations—submerged mold amylase as a saccharifying agent. Ind. Eng. Chem. **41**: 100–103 (1949).
6. Olson, W. J., Evans, Ruth, and Dickson, A. D. A corrected unit of alpha-amylase activity. Cereal Chem. **24**: 299–300 (1947).
7. Sandstedt, R. M., Kneen, E., and Blish, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723 (1939).
8. Schwimmer, S. The role of maltase in the enzymolysis of raw starch. J. Biol. Chem. **161**: 219–234 (1945).
9. Tsuchiya, H. M., Corman, J., and Koepsell, H. J. Production of mold amylases in submerged culture II. Factors affecting the production of alpha-amylase and maltase by certain aspergilli. Cereal Chem. **27**: 322–330 (1950).
10. Van Lanen, J. M., and LeMense, E. H. The production of fungal amylases in submerged culture and their use in the production of industrial alcohol. J. Bact. **51**: 595 (1946). Abstract.

THE DISTRIBUTION OF THIAMINE AND RIBOFLAVIN IN THE WHEAT KERNEL AT DIFFERENT STAGES OF MATURITY[1]

J. MARSHALL POLLOCK[2] AND W. F. GEDDES[3]

ABSTRACT

Kernels of hard red spring wheat harvested at several stages of maturity were sectioned to obtain a longitudinal section through the crease and a cross-section through the endosperm and germ. These were mounted, treated with alkaline potassium ferricyanide solution, exposed to ultraviolet radiation and the resulting fluorescence photographed. Appropriate filters were employed to select the requisite exciting radiation and to distinguish the fluorescence of thiochrome from that of riboflavin.

The photographs indicate that there was a somewhat more general distribution of these vitamins in the immature than in the mature kernels, although, even at the very early stages of kernel development, there was a relatively high concentration of thiamine and riboflavin in the scutellum and its epithelium, in the cells around the crease, and in the aleurone layer. During maturation, these vitamins become more extensively localized in these regions.

Numerous studies involving the analysis of commercial mill streams, experimental mill fractions and hand-dissected tissues have shown that thiamine and riboflavin are largely concentrated in certain structures of the wheat kernel. More precise information on the location of these vitamins has been obtained by Somers *et al.* (14) who photographed longitudinal and cross-section surfaces of wheat kernels to record the fluorescence of riboflavin and thiochrome, an oxidation product of thiamine. The sections were treated with an alkaline solution of potassium ferricyanide, exposed to ultraviolet radiation and the fluorescence of the two substances distinguished with suitable light filters. This unique method revealed that the thiamine of the wheat grain is located mainly in the scutellum and its epithelium, in the aleurone layer, and in the endosperm cells adjacent to the embryo and also those at the base of the crease. In all cases in which cellular structure could be distinguished, the thiamine appeared to be in, or adjacent to, the cell walls. This photographic technique showed the riboflavin to be much more uniformly distributed in these kernel structures than was thiamine. In general, these observations are in good agreement with the results of milling and dissection studies.

[1] Manuscript received April 20, 1951. Paper No. 2661, Scientific Journal Series, Minnesota Agricultural Experiment Station. Presented at Annual Meeting, May, 1951.

[2] This paper is based on a thesis presented to the Graduate School of the University of Minnesota by the senior author in partial fulfillment of the requirements for the M.S. degree, November, 1949.

[3] Professor of Agricultural Biochemistry, University of Minnesota, Saint Paul 1, Minnesota.

The question arises as to why these water-soluble vitamins should be distributed in the wheat kernel in this manner.

Sherwood, Nordgren and Andrews (12), from their studies of commercial mill fractions, postulated that the distribution of thiamine in the wheat kernel may be mainly due to conditions which prevail during the maturation period. Since the kernel has a high moisture content during the milk stage and thiamine is readily water soluble, they suggested that its distribution might be more uniform during that period. Water moving from inside to the surface during kernel ripening could then cause a translocation of the vitamin, and its concentration at the cell layers surrounding the endosperm. This hypothesis presumably would apply in its essentials to riboflavin, although the latter is less soluble.

Hinton (4) has pointed out that the scutellum may be considered the first leaf of the plant, and therefore, constitutes meristematic or actively dividing tissue as the embryonic plant is formed. Since such tissues are active users of thiamine, the resulting concentration gradient would cause its movement to the scutellum. When development of the embryo is complete, the scutellum passes out of the meristematic condition, and the thiamine would no longer be used; it therefore accumulates. It was further suggested by Hinton that thiamine in the scutellum is probably changed into a non-diffusible form, because it can be extracted only by subjecting the cells to a treatment which alters protoplasm permeability or denatures a protein-vitamin complex. The concentration of free thiamine would thus be low, and movement of the vitamin due to a concentration gradient would continue.

The mechanism of Hinton (4) implies that thiamine, at least, would be distributed in immature wheat kernels in a manner similar to that which has been found for mature kernels. The ideas of Sherwood, Nordgren and Andrews (12) on the other hand, would require a uniform distribution in the early stages, becoming non-uniform as maturation proceeded.

Previous researches have dealt exclusively with the distribution of thiamine and riboflavin in the mature wheat kernel. The present investigation, employing a photographic technique similar to that of Somers *et al.* (14), was undertaken to ascertain the distribution of these vitamins at various stages of kernel development.

Materials and Methods

Collection and Preparation of Wheat Kernels: To provide a supply of kernels of known maturity, 300 heads of hard red spring wheat (Rival) were tagged just after fertilization, a stage which can be identified

by the extruded anthers. Five heads each were harvested at 8, 16, 18, 21, 28 and 31 days after tagging. The kernels from the central sections of two of the heads were counted promptly into tared dishes which were then weighed before and after heating in a vacuum oven for four hours at 100°C. From these data the moisture content and the green and dry weight per 1,000 kernels were calculated to serve as an index of maturity.

For photographing the fluorescence, three kernels were selected at each stage of development to obtain a longitudinal section through the crease and cross-sections through the endosperm and the germ. Each kernel was held for sectioning by inserting it between the two halves of a pith stalk which was split lengthwise with a razor blade for a distance of about 2 cm. Free-hand sections were carefully made under a dissecting microscope with a sharp razor blade. The truncated kernels were then mounted on glass slides using Duco cement with the plane of the section parallel to the surface of the slide. With a camel's-hair brush, the sections were treated lightly with a freshly prepared solution made by mixing one volume of 0.5% potassium ferricyanide solution with five volumes of 5% sodium hydroxide solution. The fluorescence of the sections was photographed one hour after treatment.

Photographing the Fluorescence: To distinguish the fluorescence due to thiochrome from that due to riboflavin it is necessary to use two sets of filters, namely: (1) "light source" filters to provide narrow bands of radiation which will excite thiochrome and riboflavin fluorescence respectively; and (2) viewing filters to transmit the desired fluorescence of the one substance, while absorbing the fluorescence of the other as well as any reflected exciting radiation. The following filters were selected on the basis of their spectral transmission curves and available data in the literature (1,2,6,7,8,10,11) on suitable wavelength ranges for the radiation required to excite the fluorescence of the respective compounds, as well as the approximate maxima of the fluorescent spectra:

	Corning Filter No.	
	For Thiochrome	*For Riboflavin*
For Exciting Radiation	587	038+511
For Fluorescence	038+502	348

The spectral characteristics of these filters, as well as the approximate wave length ranges and positions of maximum fluorescence for thiochrome and riboflavin, are shown in Figs. 1 and 2, respectively.

The exciting radiation was provided by a General Electric A H-4 mercury vapor lamp, equipped with a 0.25 inch aperture, and a condensing lens with the requisite filters mounted in an adjustable

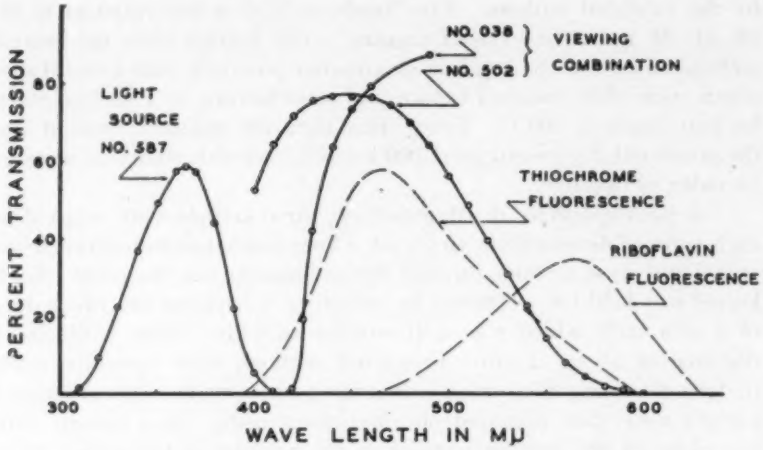


FIG. 1. Transmission spectra of Corning filters selective for thiochrome fluorescence. The approximate fluorescent spectra of thiochrome and riboflavin are shown in broken lines.

carrier by means of which the maximum intensity of light could be focussed in the plane of the kernel section at an angle of 35 to 40°. The specimens were mounted upon a vertical stage, backed by black paper, and the fluorescence was photographed with a Leitz Photomicrographic camera equipped with a Leitz Summar lens (using an aperture equivalent to f4.5 and a magnification of approximately 10×). The viewing filters were placed between the lens and the film. Since the thiochrome fluorescence was quite brilliant and is

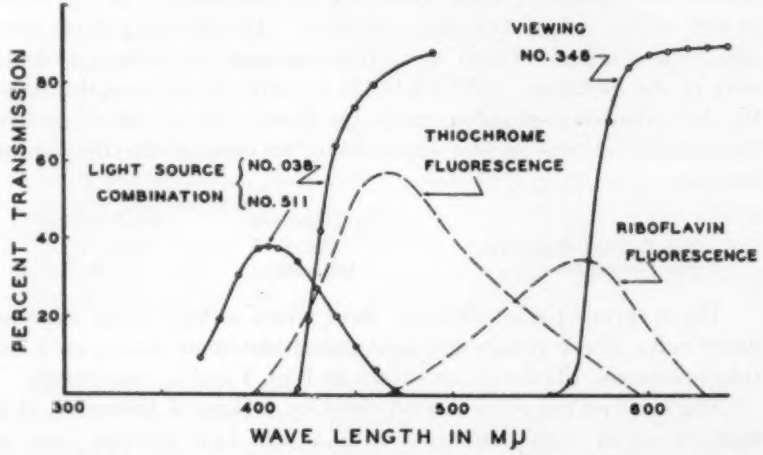


FIG. 2. Transmission spectra of Corning filters selective for riboflavin fluorescence. The approximate fluorescent spectra of thiochrome and riboflavin are shown in broken lines.

composed of photographically active wave lengths, it was possible to use a 15-second exposure on a fast film (Eastman Super Ortho Press), thereby minimizing the destruction of thiochrome by ultraviolet radiation. The riboflavin fluorescence which passed the viewing filter, on the other hand, was relatively slight and also weak in its action on ordinary film; accordingly, to obtain a satisfactory image, Super Panchro Press B, a film of extra color sensitivity, was used with a 5-minute exposure.

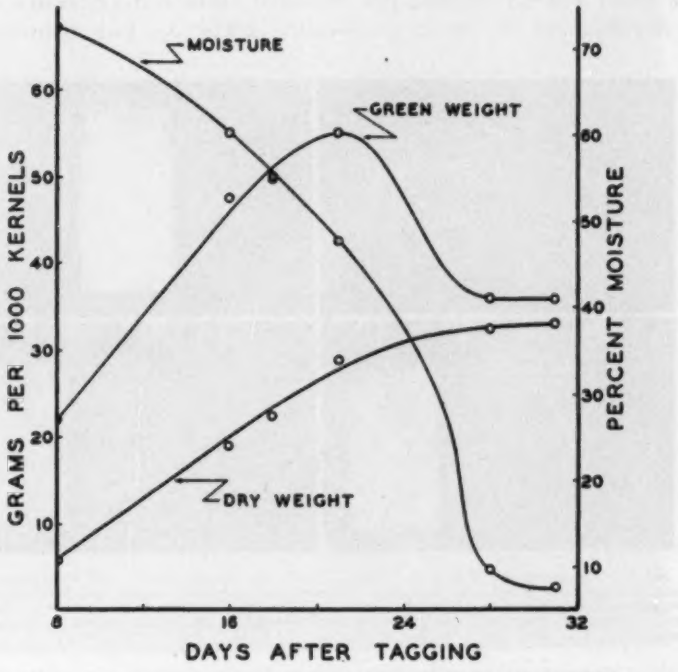


FIG. 3. Progressive changes in moisture content, green weight, and dry weight of wheat kernels during the course of maturation.

To ascertain the selectivity of the filter combinations, the method of Somers *et al.* (14) was used. Strips of filter paper treated with distilled water and with 0.001 *M* solutions of thiamine and riboflavin, respectively, were photographed before and after treatment with the alkaline potassium ferricyanide reagent, using the filter combinations and photographic techniques described above. The effect of the reagent upon thiochrome and riboflavin fluorescence in the kernel sections was also determined, by photographing a cross section through the germ before and after reagent application, again employing

the filters and methods previously described. To learn whether any appreciable time was required for the full development of fluorescence, a single longitudinal section was photographed immediately after reagent application and at intervals thereafter, using alternately the filters and films selective for thiochrome and riboflavin fluorescence.

Results and Discussion

Development of Kernels: The mean values for moisture content and for the green and dry weights per thousand kernels at successive stages of development are shown graphically in Fig. 3. Low temperatures

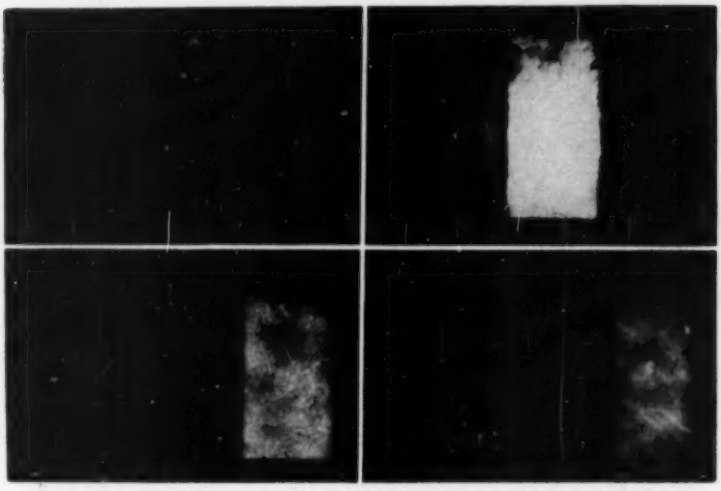


FIG. 4. Determination of filter selectivity. Each photograph depicts three filter paper strips, impregnated with, left to right, distilled water, thiamine hydrochloride solution, and riboflavin solution. Photographs in top row taken using "thiochrome" filters; those in bottow row taken with "riboflavin" filters. The two photographs to the right have been treated with alkaline ferricyanide. Magnification approx. 7 ×.

and liberal rainfall early, followed by hot dry weather later in the growing season, resulted in rapid desiccation of the kernels to a moisture content of 7.8% at maturity.

Evaluation of Method: Figure 4 illustrates the results obtained in testing the selectivity of the methods with filter paper strips. While the filters used were quite selective for the fluorescence of thiochrome and riboflavin, some fluorescence is contributed by the filter paper, especially with the filters used for thiochrome.

The effect of the alkaline ferricyanide solution on the fluorescence of a transverse section of a mature wheat kernel is shown in Fig. 5. Since wheat contains very little cocarboxylase (9), no attempt was

made to distinguish thiochrome from the oxidized form of cocarboxylase which Somers *et al.* (14) have shown to fluoresce in the same manner as thiochrome. There is a very faint fluorescence of the untreated section when employing the filters for photographing the fluorescence due to thiochrome, but the interference is not serious. The filters selected to detect riboflavin record little, if any, fluorescence in the untreated endosperm. Somers and Coolidge (13) made a

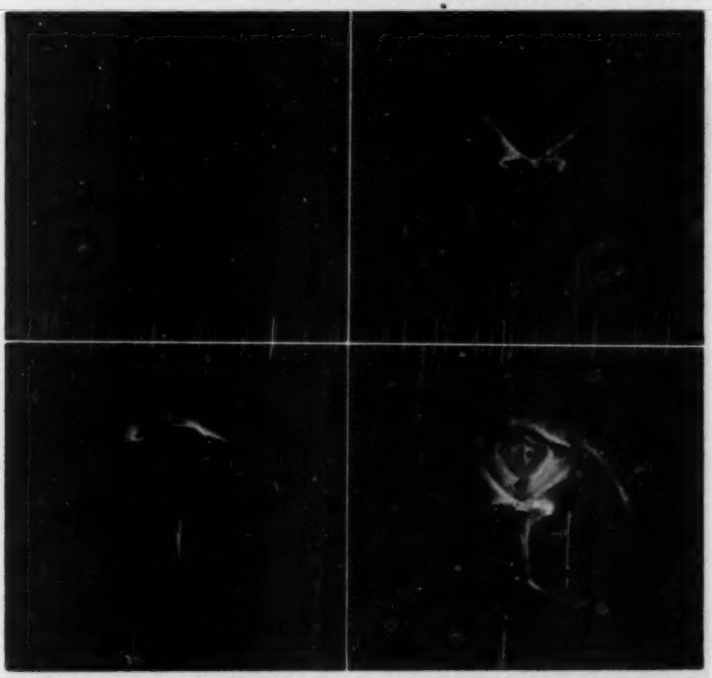


FIG. 5. Effect of the alkaline ferricyanide reagent on the fluorescence of thiochrome and riboflavin in a transverse section of a mature wheat kernel. *Upper left:* Untreated section photographed using "thiochrome" filters. *Upper right:* Treated section photographed using "thiochrome" filters. *Lower left:* Untreated section photographed using "riboflavin" filters. *Lower right:* Treated section photographed using "riboflavin" filters. Magnification 10 ×.

similar observation which they attributed to the combination of riboflavin in a protein complex, which is dissociated upon treatment with alkali. In the present study there is a marked fluorescence in the bran layer of the untreated kernels, which was not apparent in the photographs of Somers *et al.* (14).

Photographs taken of thiochrome and riboflavin fluorescence at different intervals of time after the addition of alkaline ferricyanide solution indicate that thiochrome fluorescence reaches its maximum

in 15 minutes or less, while riboflavin attains its maximum fluorescence in 7 minutes or less. In neither case was there evidence of the migration of fluorescent substances due to the reagent solution.

Distribution of Vitamins at Different Stages of Maturation: Photographs of longitudinal and transverse sections of wheat kernels taken 16 and 21 days after blossoming and at maturity (31 days) are shown in Figs. 6, 7 and 8 respectively. At 16 days, the thiochrome and riboflavin fluorescence are both quite weak but localization of the vitamins is evident even at this early stage. Geddes and Levine (3)

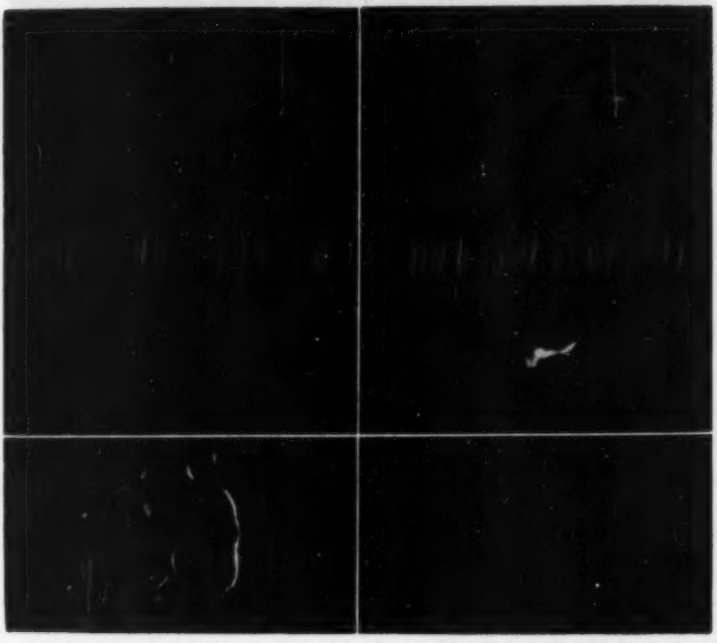


FIG. 6. Photographs of wheat kernel sections sixteen days after blossoming treated with alkaline ferricyanide solution. *Left:* Thiochrome fluorescence. *Right:* Riboflavin fluorescence. Magnification 10 ×.

have shown that the quantity of thiamine present in the kernels increases steadily during maturation, at the expense of that present in other parts of the plant. Similarly, Hoffer (5) has shown that the total riboflavin content of the kernels increases during the early stages of kernel development but later decreases. Comparison of Fig. 7 with Fig. 6 shows that while the amounts of thiamine and riboflavin have markedly increased between the 16th and 21st days, there is no marked change in their distribution within the kernel.

This is not surprising since Fig. 3 shows that the dry matter weight of the kernels was increasing rapidly during this period. However, some desiccation occurred. Between the 21st day and maturity, however, a notable change in the distribution of the vitamins occurred concurrently with a large decrease in moisture content. The distribution of thiamine is more highly concentrated in the cells around the crease and adjacent to the germ. In the endosperm, the fluorescence assumes the appearance of a network which apparently corresponds with the cell walls. It thus appears that the thiamine becomes lodged at the cell walls during kernel desiccation.

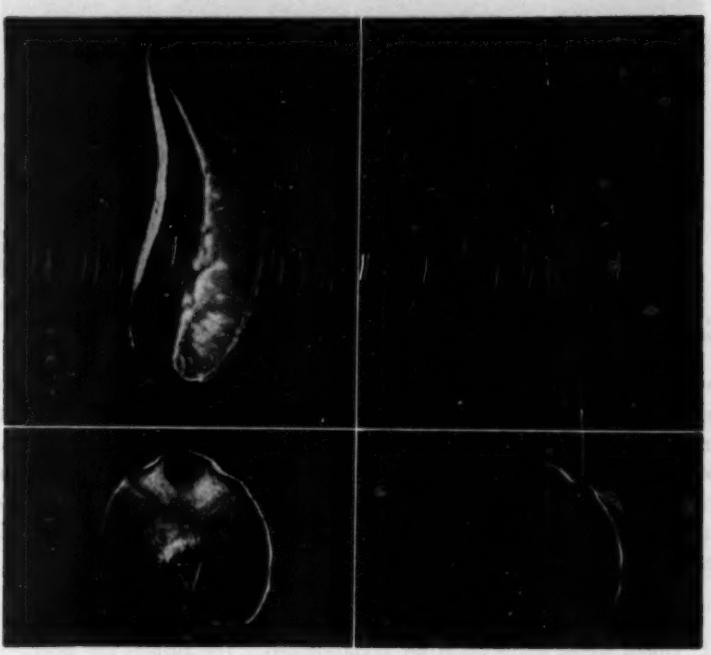


FIG. 7. Photographs of wheat kernel sections twenty-one days after blossoming treated with alkaline ferricyanide solution. *Left:* Thiochrome fluorescence. *Right:* Riboflavin fluorescence. Magnification 10 ×.

Riboflavin appears to undergo a type of redistribution similar to that of thiamine. The outstanding feature is the comparatively heavy concentration in the germ, which appears to be laid down primarily during the late maturation period.

Considering the photographs as a whole, there appear to be certain points of thiamine and riboflavin concentration which are independent of the maturity of the kernel, namely the aleurone layer, the cells around the crease, and the scutellum and its epithelium, but additional

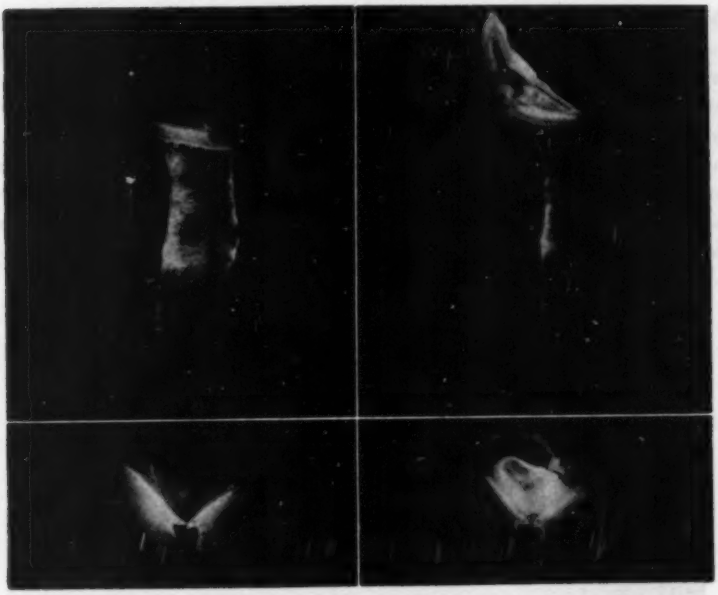


FIG. 8. Photographs of wheat kernel sections thirty-one days after blossoming (maturity) treated with alkaline ferricyanide solution. *Left:* Thiochrome fluorescence. *Right:* Riboflavin fluorescence. Magnification 10 ×.

amounts of the vitamins are more uniformly distributed in the early stages. These additional quantities of vitamins appear to be relocated during maturation, although the mechanisms by which thiamine and riboflavin are redistributed do not seem to be identical. The results give some support to the theory of Sherwood *et al.* (12) that these vitamins, by virtue of their water solubility, are translocated during the course of maturation.

Acknowledgments

The photographs were made in the University Photographic Laboratory by V. P. Hollis, whose aid is gratefully acknowledged. The authors are indebted to C. M. Christensen, Division of Plant Pathology, for suggestions concerning sectioning techniques.

Literature Cited

1. BARGER, G., BERGEL, F., and TODD, A. R. A crystalline fluorescent dehydrogenation product from vitamin B_1. Nature **136**: 259 (1935).
2. BOOHER, LELA E. Chemical aspects of riboflavin in The Vitamins, pp. 249–270. American Medical Assoc., Chicago (1939).
3. GEDDES, W. F., and LEVINE, M. N. The distribution of thiamin in the wheat plant at successive stages of kernel development. Cereal Chem. **19**: 547–552 (1942).
4. HINTON, J. J. C. The distribution of vitamin B_1 and nitrogen in the wheat grain. Proc. Roy. Soc. (London) **134B**: 418–429 (1947).
5. HOFFER, A. Studies on the B vitamins of wheat. Ph.D. Thesis—The University of Minnesota (1944).

6. KUHN, R., and VETTER, H. Zur Kenntnis des Thiochroms. Ber. **68B**: 2375–2385 (1935).
7. LOEWENSTEIN, E. Glass standards of fluorimetric determinations. Ind. Eng. Chem. (Anal. Ed.) **15**: 658 (1943).
8. LOY, H. J. Chemical assay for riboflavin in Biological Symposia **12**: 166–180 (1947).
9. OBERMEYER, H. G., FULMER, W. C., and YOUNG, J. M. Cocarboxylase hydrolysis by a wheat phosphatase. J. Biol. Chem. **154**: 557–559 (1944).
10. ROSENBERG, H. R. Chemistry and physiology of the vitamins. Interscience: New York (1942).
11. ROSENBERG, H. R. Vitamins, vitagens and hormones in The Chemistry and Technology of Food and Food Products (ed. by M. B. Jacobs), Vol. I, pp. 229–269. Interscience Publishers, Inc., New York (1944).
12. SHERWOOD, H. C., NORDGREN, R., and ANDREWS, J. S. Thiamin in the products of wheat milling and in bread. Cereal Chem. **18**: 811–819 (1941).
13. SOMERS, G. F., and COOLIDGE, MARY H. Location of thiamin and riboflavin in wheat grains. Science **101**: 98–99 (1945).
14. SOMERS, G. F., COOLIDGE, MARY H., and HAMNER, K. C. The distribution of thiamine and riboflavin in wheat grains. Cereal Chem. **22**: 333–340 (1945).

MODIFICATION OF THE BUHLER MILL FOR MICRO MILLING [1,2]

E. F. SEEBORG, N. H. SHOUP, and M. A. BARMORE

ABSTRACT

A scaled down feeder to fit the grinding rolls of the Buhler laboratory flour mill and designed to give reliable milling quality data from 100 g. samples of wheat is described. The method of operation and some preliminary results with five common soft white wheats of differing milling qualities are presented and they indicate that the feeder and method as described are satisfactory for the purpose of detecting poor milling quality earlier in the breeding program than was heretofore possible.

One of the most pressing problems of the plant breeder is that of producing smut and rust resistant varieties with other desirable agronomic properties. Naturally the most resistant variety of good agronomic properties would be used extensively in making new hybrids, but since that variety may have very undesirable milling characteristics, tests for these are extremely important. One of the most smut resistant varieties in the Pacific Northwest is Rex and this variety is also the most troublesome in milling ever encountered commercially, hence the emphasis on "milling quality" as an important factor in the wheat breeding program. The fact that some other parent material used may also be undesirable from the milling standpoint, usually to a lesser degree than Rex, makes the determination of the milling quality

[1] Manuscript received January 8, 1951.
[2] Contribution from the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations; the Division of Industrial Research, Washington State Institute of Technology, The State Sollege of Washington; and the Division of Cereal Crops and Diseases, Bur. of Plant Ind., Soils and Agr. Eng., Agriculture Research Administration, U. S. Department of Agriculture.

essential in the early characterization of new varieties. Although work is under way to learn the causes of these troublesome problems and to develop chemical, microscopic, or grinding and sifting tests, no procedure other than "a milling test" is available at present to determine these characteristics. Seeborg (8) has described a satisfactory milling modification for use with the Buhler Experimental mill, using a 2400 g. sample of grain, a modified flow and a technique designed to magnify the undesirable milling properties. However, the time, plot space, labor and expense involved to furnish the laboratory with sufficient seed for such a trial milling has made the search for a test using only 100 g. of grain one of immediate importance.

It is necessary that a small scale milling test produce milling fractions typical of those produced commercially so that differences in bran, shorts, middlings, and flour characteristics can be observed and recorded. It is in some of these almost intangible differences that the differences in milling quality of soft wheat varieties are found. A slight fluffiness in the middlings can be the clue to a very undesirable characteristic which translated to a commercial mill, can mean hundreds of dollars a day in lost milling capacity.

Geddes and Frisell (5) and Harris and Sanderson (6) described a scaled-down Allis mill which they claim is satisfactory for milling 100 g. of grain. Finney and co-workers (2, 3), and McCluggage (7), using a Hobart grinder, produced flour having baking properties similar to commercially and Buhler experimentally milled flours. Others have developed micro milling techniques to produce flour or semolina from small samples of grain (4) but not to determine the milling quality. The Allis micro milling technique and the Hobart micro method were investigated using western soft white wheats of known difference in milling behavior, but neither one gave as accurate a differentiation as was desired. This was probably partly due to the use of silk and wire bolting and scalping cloths unsuitable for soft wheat. It became evident that the methods described for the micro milling of hard wheats would not be applicable "in toto" to the characterization of soft wheats. Sifting studies of ground meal by Butler (1) resulted in some remarkable correlations with a milling quality rating obtained by regular milling trials, but some important exceptions could not be eliminated.

Mill Modification

Since none of the available micro methods appeared to characterize Pacific Northwest varieties adequately from the milling standpoint it was considered worthwhile to alter the Buhler mill for micro milling. The Division of Industrial Research of Washington State Institute of

Technology agreed to undertake this task. The requirements, as set forth, were as follows:

1. Reduce milling loss to a minimum.
2. Reduce exposed length of the break and reduction rolls in proportion to the size of the sample to be milled.
3. Correspondingly reduce working length of feed rolls and spreader blade surfaces while retaining normal feeding action.
4. Modify the sifting section of the mill if necessary, to permit handling of small samples.
5. Accomplish all modifications so that the change over from macro- to micro-milling can be made conveniently and quickly.

To reduce milling loss, it was decided to eliminate the elevators. (See Fig. 1—elevators in inoperative position in background.) Since the elevators and feeders are incorporated in one unit in the Buhler mill, the most convenient modification was to construct an entirely separate feeder of the desired design. Laying the elevator section back, as shown in Figs. 1 and 2, in an inoperative position exposed the mill fraction outlets of the sifter box and made them accessible for collection of individual milling fractions at the rear of the mill. However, milling trials forced the decision to accomplish the complete sifting operation outside the mill on a laboratory shaker. This necessitated construction of metal trays to be placed directly under the grinding rolls to facilitate removal of the mill fractions. As a consequence,

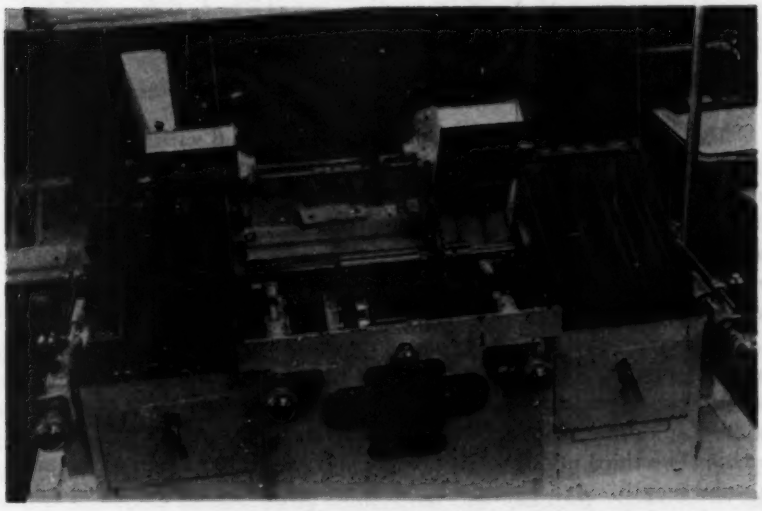


FIG. 1. View of top of Buhler experimental mill with micro feeder in place and the macro feeder and elevator assembly laid back.

no modifications were made on the sifting box. Requirements 1 and 4 were then satisfied by employing the milling arrangement illustrated in Fig. 2.

The method of assembly of the new feeder is illustrated in Fig. 3, which is an exploded view of the reduction section of the feeder. To form the feeder sections of reduced width, one-eighth inch aluminum sheet was cut to approximate shape and assembled as a stack of 15 plates. The stack was then clamped and machined to insure proper alignment of the sides, holes for the feed rolls, and the slots and projections for welding.

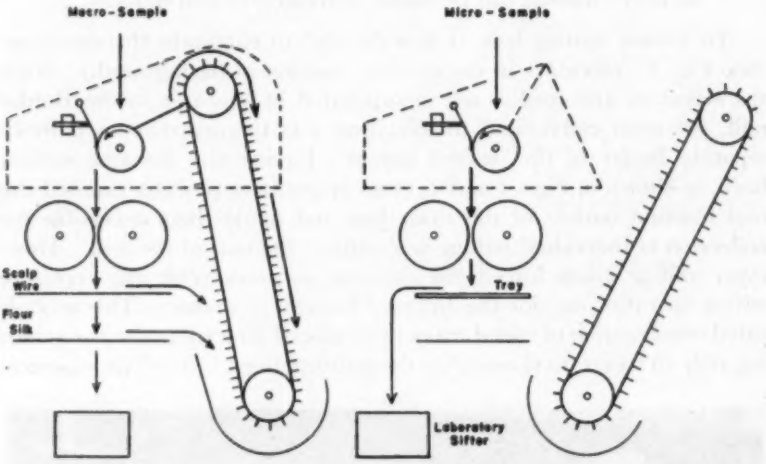


FIG. 2. Schematic diagram of macro and micro Buhler experimental milling equipment.

The front and back spacer plates were provided with slots to properly align the division plates, as shown. The division plates were also slotted to allow assembly of the insert plate which forms a feeder bin for each section. The slotted and mating plates were designed so that all welds could be made with a heliarc welding torch on the exterior surfaces of the feeder. The welds were then ground to obtain a smooth surface.

The completed feeder gives redimensioned break and reduction sections in the following lengths: first break, one and one-half inches; second break and first middling, three-fourths inch; third break and second middling, nine-sixteenth inch; and third middling, one-half inch. The third middling section was not a full reduction to one-fifth normal length. It was considered, however, to be the practical minimum length for satisfactory operation. The division plate assemblies

for the grinding rolls were made to correspond with the above dimensions to fulfill the 3rd requirement, that of reducing the exposed length of the grinding rolls. This assembly, as shown in Fig. 3, fits directly over the grinding rolls and immediately below the feeder.

The drive sprocket, coupling, and feed rolls were machined from steel. Care was taken to duplicate the feed rolls as to size and surface. The feed rolls are supported by ball bearings housed in the bearing caps machined from magnesium stock (see Fig. 1). The wheat-feeder-slot was made adjustable by a gate controlled by a cali-

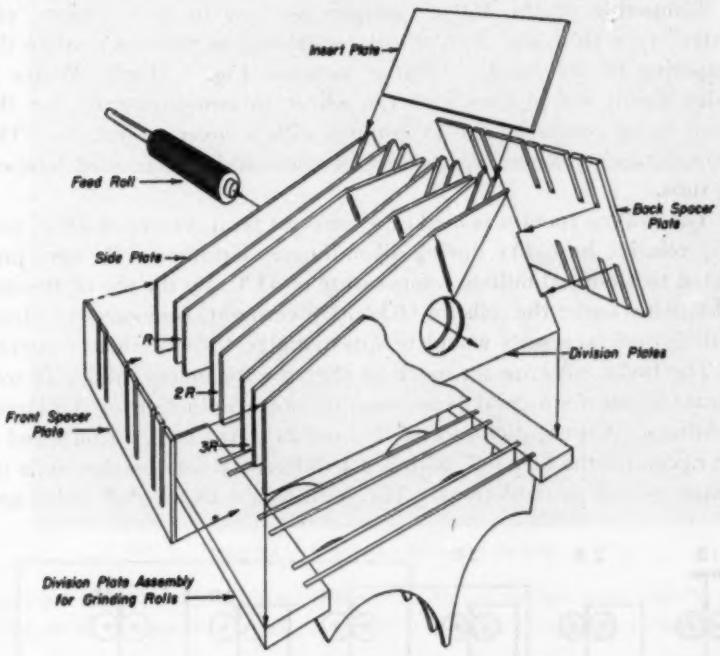


FIG. 3. Exploded view showing micro feeder construction.

brated knob. Some modifications of the original design are incorporated in this feature, but the feeder-slot is the same size as the original. To improve feeding of wheat through the slot a striker pin was installed on the feed roll. The spreader blades, illustrated diagramatically in Fig. 2., were fashioned from sheet steel and provided with adjustable brass counter weights. It was found that the reduced width spreaders satisfactorily duplicated the operating characteristics of the Buhler spreader blades.

The milling system enables the changeover from macro to micro milling to be made in less than an hour by one man with little inconvenience to the normal laboratory arrangement. All that is required is replacement of the feeder and elevator assembly by the micro-feeder and disconnection of the sifter box belt. Figure 1 gives a view of the feeder in place and ready for use.

Micro Milling Procedure

One hundred grams of clean wheat tempered to 14% moisture for 72 hours is milled through the flow illustrated in Fig. 4.

Tempering of the 100 g. samples is done in scaled-down "pill coater" type tubs, Fig. 5 (bottom), modeled after the ones used for the tempering of the regular 2,400 g. samples, Fig. 5 (top). Water is added slowly by pipette with the wheat in motion, turning of the wheat being continued for 15 minutes with a cover on the tub. The same motors and assembly are used for micro and regular-sized tempering tubs.

The milling room was held at a constant temperature of 28°C. and 60% relative humidity during all millings. Grinding rolls were preheated to a normal milling temperature of 33°C. by the use of 40-watt light globes under the rolls for 16 hours (overnight) previous to milling. Milling quality results would be questionable without these controls.

The break rolls are set much as they are for macro milling of soft wheats to obtain a good bran cleanup and a reduction of the larger middlings. Corrugations are 16, 20, and 24 to the inch. Roll speed is 535 r.p.m. on the fast roll, with a 2:1 differential for the slow rolls on breaks as well as reductions. The reduction rolls are dull finish and

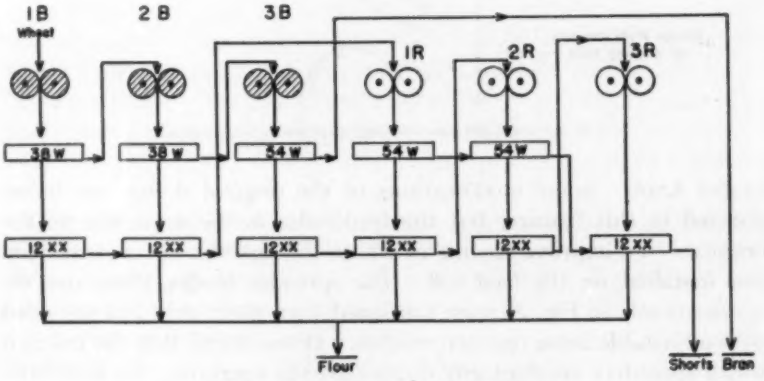


FIG. 4. Buhler micro milling flow sheet.

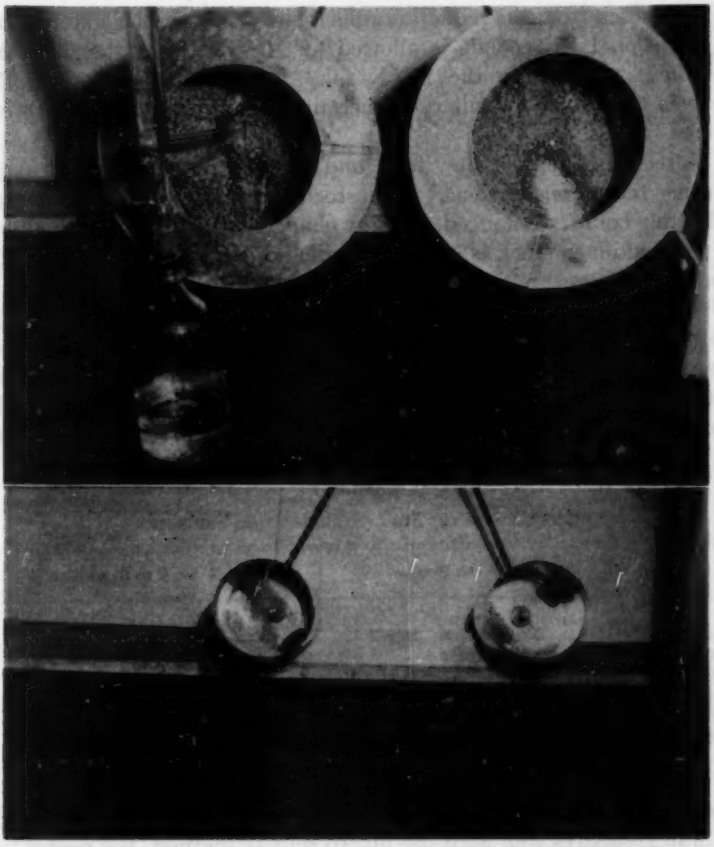


FIG. 5. Tempering tubs (top) for 2,400 g., and (bottom) for 100 g.

are set slightly closer together in order to get satisfactory results with the small amounts of stock handled. Those settings are as follows:

Breaks			Reductions		
1B	2B	3B	1R	2R	3R
.47 mm	.05 mm	.03 mm	.04 mm	.023 mm	.006 mm

The scalping and sifting is done on a 14-in. square laboratory shaker running 220 r.p.m. The stock from the break rolls is caught in trays directly under the rolls and is sifted exactly 20 seconds over the scalping wire. This material, comprising the stock to the next break, or finally bran, is removed from the sifter box. The remaining middlings and flour are sifted for an additional 40 seconds on the 12XX silk. Five seconds before the end of the sifting time, the sieve is tapped with a rubber hammer to jar the flour from the underside.

The stock from the reduction rolls is sifted in a similar manner, but an additional 15 seconds is allowed for the sifting of the flour and middlings mixture on the 12XX silk—a total of 75 seconds. The sieve is tapped in a similar manner to that employed for the break stocks.

A record is kept of the "feel" and amount of the stock fed to the various reduction sections. The stock spreaders or feed gates are delicately counter-balanced, and any change in granulation or fluffiness of the middlings stock is reflected in the adjustments necessary in the feed gates.

Visual comparisons indicate that the bran fractions obtained by the micro and macro milling procedures are entirely comparable. Such characteristics as cleanup, toughness and brittleness, are readily noticeable and are the same in both methods of milling. A similar comparison of the shorts streams show that the micro method magnifies the poor milling qualities of the samples. While the percentage of bran compares well with the macro milling method, the percentage of shorts is usually higher with greater differences between varieties.

TABLE I

COMPARISON OF MILL STREAMS FOR VARIETIES

Variety	% Break Release			% Stock to Reductions		
	1B	2B	3B	1R	2R	3R
Golden	25.6	24.1	3.2	49.7	22.1	9.2
Orfed	25.7	23.7	3.0	49.4	23.2	12.9
1-3-12	22.9	21.6	3.2	44.5	22.6	13.2
Marfed	23.5	27.2	3.2	50.7	25.0	14.1
Rex	18.3	24.2	3.6	42.4	23.1	15.1

Several characteristics of good and poor milling varieties are shown by the results in Table 1. Good milling varieties, as indicated by Golden, and to a lesser extent, by Orfed, yield a larger percentage of middlings going to the first reduction than do the poorer varieties 1-3-12 and Rex. This is related to the large amount of break flour produced by the latter varieties. However, the variety Marfed is an exception to this general pattern in that it produces about the same amount of total middlings as Golden, the difference being in the characteristics of the middlings. The poor milling quality of Marfed is due primarily to the fluffy nature of its middlings, causing a larger percentage to tail over into shorts.

The weight of middlings remaining after two reductions and siftings is a significant characteristic of each variety since it serves as a

measure of the comparative rates of milling. The relative rank of the varieties tested in this series is the same as that obtained in previous millings by the macro procedure.

Yields shown in Table 2 were calculated from the formula 100-(Bran + Shorts) and are on an "as is" basis. The milling losses were too irregular to warrant use of the actual flour recovery in the calculations.

Ash determinations on the flour (Table 1) obtained by this micro method indicate that the flour corresponds to an 85% patent. The amount of flour is limited but there is sufficient for viscosity and mixer curve area determinations. To have sufficient flour for bread or

TABLE II

COMPARISON OF MICRO AND MACRO MILLING DATA[1]

Variety	Bran		Shorts		Flour Yield[2]		Ash[3]	
	Micro	Macro	Micro	Macro	Micro	Macro	Micro	Macro
	%	%	%	%	%	%	%	%
Golden	19.7	20.9	10.7	6.5	69.6	72.6	0.37	0.34
Orfed	19.9	20.5	12.5	10.5	67.8	69.0	0.32	0.31
1-3-12	21.5	20.3	12.3	11.2	66.2	68.5	0.33	0.32
Marfed	18.3	18.1	14.7	15.4	67.0	66.5	0.35	0.33
Rex	22.8	20.4	17.1	14.7	60.2	64.9	0.36	0.35

[1] Micro milling data average of three replications and macro single determinations.
[2] 100 − (% Bran + % Shorts).
[3] For 85% patent flour.

cookie baking, it would be necessary to mill 200 g. of wheat. If only baking information is sought, modification of the sifting technique and flow could control the ash content to almost any desired level.

Comparison of the micro yields with those for the macro method given in Table 2 indicates that the characteristic of low flour yield of the extremely poor milling varieties is magnified by the micro method. Otherwise, the comparative yields are in fair agreement with macro milling studies. The flour loss during milling is relatively high with the micro method.

Acknowledgments

The courtesy extended to Mr. Seeborg by Dr. R. H. Harris and Mr. L. D. Sibbit of the North Dakota Agricultural Experiment Station, Fargo, North Dakota, in arranging for a special milling of soft white wheats under his observation on the micro mill in their laboratory is gratefully acknowledged. Similar credit is due to Mr. C. E. Bode, and H. K. Heizer, Federal Soft Wheat Quality Laboratory, Wooster, Ohio, for their cooperation in milling the same wheats on their Hobart mill under Mr. Seeborg's observation.

Literature Cited

1. BUTLER, ROBERT J. Unpublished results of the correlation of a sifting technique on small samples of Hobart ground wheat with milling quality (1950).
2. FINNEY, K. F., and YAMAZAKI, W. T. A micro-milling technique using the Hobart grinder. Cereal Chem. **23**: 484–492 (1946).
3. FINNEY, K. F., HEIZER, H. K., SHELLENBERGER, J. A., BODE, C. E., YAMAZAKI, W. T. Comparison of certain chemical, physical, and baking properties of commercial, Buhler, and Hobart-milled flours. Cereal Chem. **26**: 72–80 (1949).
4. FISHER, M. H., and MEREDITH, W. O. S. Note on a semimicro milling procedure for durum wheat. Cereal Chem. **23**: 565–568 (1946).
5. GEDDES, W. F., and FRISELL, B. An experimental flour mill for 100-gram wheat samples. Cereal Chem. **12**: 691–695 (1935).
6. HARRIS, R. H., and SANDERSON, T. A comparison between the Allis-Chalmers and micro-milling techniques on North Dakota hard red spring wheats. Cereal Chem. **16**: 619–625 (1939).
7. MCCLUGGAGE, M. E. Micro milling and baking of small samples of wheat. Cereal Chem. **20**: 185–193 (1943).
8. SEEBORG, E. F. A modified flow sheet for Buhler mills. Northwestern Miller **241** (2): 1a, 21a–22a (1950).

AMYLOSE CONTENT OF SWEETPOTATO STARCH[1]

GORDON L. DOREMUS,[2] FRANK A. CRENSHAW, and F. H. THURBER

ABSTRACT

The amylose content of starches prepared from 22 varieties of sweetpotatoes varied from 17.5 to 21.7% as determined by potentiometric iodine titration. The majority of the values ranged from 20.0 to 21.7%; and the average was 20.4%. In no instance was there a sufficient trend toward high amylose content to make the variety of interest in selection or breeding for high amylose starch.

The percentage of iodine bound by sweetpotato amylose of the highest purity was 19.3.

Breaks in the horizontal sections of the iodine sorption curves suggest the presence in sweetpotato starch of small amounts of a fraction intermediate between amylose and amylopectin in degree of branching or the presence in the amylopectin fraction of external branches sufficiently long to bind appreciable quantities of iodine at higher free iodine concentrations.

A closed titration chamber for use in making potentiometric iodine titrations is described.

A procedure is given for the laboratory preparation of starch from sweetpotatoes.

Demonstration of the properties and potential industrial uses of amylose, the straight chain component of starch which can be separated by fractionation, has stimulated interest in possible sources of the material in commercial quantities. However, starches of present industrial significance, such as corn starch, white potato starch, and

[1] Manuscript received October 16, 1950. Contribution from the Southern Regional Research Laboratory, New Orleans, Louisiana. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Resigned December 1, 1947.

tapioca starch contain only 20–30% amylose (6) and fractionation by methods now available does not appear to be economically feasible. As an alternative, attention has been directed toward a search for a "high amylose" starch which can be produced in industrially significant quantities. The fact that starches are known which are composed entirely of amylopectin, i.e., the "waxy" cereal starches, and that starch from wrinkled seeded peas contains as much as 70% amylose, indicates that there is some possibility of success for such a program. Sweetpotato starch has been stated to contain about 20% amylose (3) but analysis of starch from the different strains and varieties of sweetpotatoes to determine the extent of variation in the amylose content has not previously been reported.

In the present work starches from 22 varieties of sweetpotatoes, including both starch and tablestock types,[3] were analyzed for amylose to provide a basis for (a) selection of any variety that might be promising with respect to amylose content for inclusion in future sweetpotato breeding programs; (b) determination of the percentage of amylose in an average sample of sweetpotato starch; and (c) comparison of the iodine-sorption characteristics of sweetpotato starches and other starches. With this latter objective in view, the amylose content of samples of white potato, corn, and tapioca starches was also determined.

Materials and Methods

Preparation of Starches. Samples of starch for analysis were extracted from the different varieties of sweetpotatoes by disintegrating 500 g. of peeled sweetpotatoes in a Waring Blendor, with distilled water as the suspension medium. The resulting slurry was screened through a 325-mesh sieve to remove most of the pulp, and the crude starch milk was centrifuged in 200-ml. bottles to separate the starch from the "fruit water," the latter being subsequently discarded. The settled starch was resuspended in distilled water and stirred for 15 minutes prior to screening through a 400-mesh sieve. After screening, the starch was collected on a suction filter, and this cycle of washing, screening, and filtration was repeated 5 times to effect a rather complete purification of the starch. The final starch cakes were dried at room temperature, powdered by rolling to pass through an 80-mesh sieve, and bottled for analysis. Some indication of their high degree of purity is afforded by the results of ash and solubles determinations, which averaged 0.016% and 0.011%, respectively. Since fatty material in the cereal starches has been shown to reduce their apparent amylose content, as determined by potentiometric titration with

[3] The sweetpotatoes used in these investigations were samples from the crop of comparable plots in a variety test conducted by the Mississippi Agricultural Experiment Station in 1946.

iodine (10), small portions of several of the sweetpotato starch samples were extracted with methanol in a Soxhlet apparatus for 20 hours to remove the very small amounts of fatty material known to be present in these starches. A sample of commercial corn starch was also extracted in this manner and the last traces of methanol were removed from these samples by leaving them in an oven at 50°–55°C. overnight. A sample of white potato starch was extracted by the sweetpotato starch procedure for use as a reference sample in developing a satisfactory analytical procedure. A good grade of commercial tapioca starch was further purified by repeated washing with distilled water followed by screening and drying.

Amylose. Sweetpotato amylose was prepared by fractionating a 2% autoclaved paste of unbleached starch extracted from sweetpotatoes of the Pelican Processor (L-5) variety. A mixture of butanol and isoamyl alcohol was used as the fractionating agent, in accordance with a procedure proposed by Wilson, Schoch, and Hudson (15) for use where a supercentrifuge is not available to collect the precipitated amylose complex. The crude amylose containing about 14% amylopectin was purified by 5 recrystallizations from hot water saturated with butanol. At this point, the supernatant solution showed no turbidity with a large excess of ethanol and the amylose was considered to be pure. It was dehydrated with methanol, then dried in a vacuum oven at 60°C. and subsequently in a desiccator over phosphorus pentoxide. This final product gave a deep blue color with iodine and bound 19.3% iodine by weight (Fig. 1). This value is comparable to those reported in the literature for other amyloses prepared by similar procedures (6, 10).

Amylopectin. Crude amylopectin was precipitated from the original starch paste after removal of the crude amylose complex by pouring the paste into approximately five volumes of absolute ethanol. The flocculated amylopectin was collected by vacuum filtration, triturated several times in the Waring Blendor with fresh portions of absolute ethanol and finally dried in vacuo over calcium chloride. This material bound only 0.83% iodine, corresponding to 4.3% amylose (Fig. 2-A).

Procedure for the Determination of Amylose. The potentiometric iodine titration of Bates, French, and Rundle (1) as modified by Wilson, Schoch, and Hudson (15)[4] was used for the amylose determination. The essential features of the procedure are (a) dispersion of the starch sample in 0.5 *N* alkali, (b) neutralization with hydrochloric acid, (c) addition of solid potassium iodide to give a final solution 0.05 *N* in both potassium chloride and potassium iodide, and (d) potentiometric

[4] A later description of the modified Bates, French, and Rundle method, with some further refinements, published since completion of the work reported herein, is given by Lansky, Kooi, and Schoch (8).

titration of this solution with 0.001 *N* iodine likewise 0.05 *N* in both potassium iodide and potassium chloride. Several features were incorporated, which while possibly not new, have not been reported in the literature. These were (a) use of a Waring Blendor to facilitate dispersion of the sample in alkali; (b) titration in a closed chamber to minimize loss of iodine during titration, and (c) close temperature control during the titration.

The closed titration chamber consists of a tubulated 200 ml. round bottom flask in which are mounted, through standard taper joints, a bright platinum electrode, a saturated calomel electrode, a thermometer graduated in 0.2°C. intervals, a stirrer equipped with a

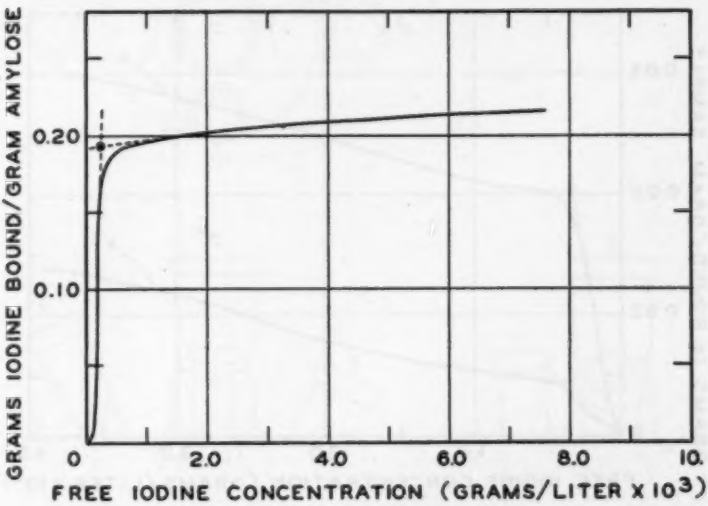


FIG. 1. The relation between the free iodine concentration and the iodine bound by sweetpotato amylose.

mercury seal, and a 25 ml. burette. The design of the flask is shown in Fig. 3. Tubulatures A and B, joint size 19/38, are for the electrodes; C, size 24/40, is for the stirrer; and D, size 14/35, is for the burette. E is a sealed-in thermometer well. It is important that a closed titration chamber be used, since the iodine loss from an open beaker may be as high as 5% of the amount added during the time required to reach the end point of a titration. The use of a closed titration chamber reduces this loss to approximately 1% of the titer. A relatively constant temperature was maintained by supporting the titration chamber in a large beaker through which water at 25° ± 0.2°C. was circulated.

Calculation of Results. The per cent iodine bound by the amylose component of the sample was calculated by the method of Bates, French, and Rundle (1). These authors plot grams iodine bound per gram starch against free iodine concentration and by extrapolating the vertical and horizontal sections of the resulting curve to a point of intersection, arrive at a value representing the iodine bound by the amylose fraction alone. The curve for a representative sweetpotato starch is shown in Fig. 2-B. The free iodine concentration at any stage of the titration is obtained from the observed oxidation potential by reference to a curve resulting from the potentiometric titration with iodine of 0.05 *N* potassium iodide-potassium chloride solution

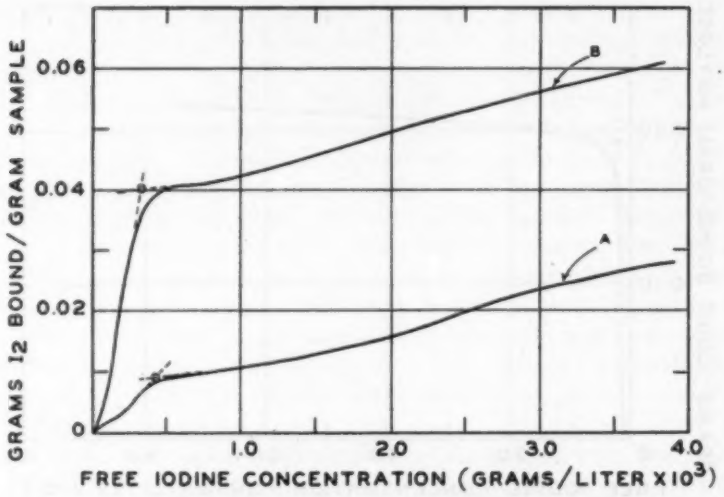


FIG. 2. The relation between the free iodine concentration and the iodine bound by (A) Sweetpotato amylopectin and (B) Sweetpotato starch, Pelican Processor variety.

alone. The percentage of amylose in the sample was calculated from the ratio of iodine bound by the sample to iodine bound by pure sweetpotato amylose.

The amylose content of the titrated sample may also be determined from the end point data alone, that is, milliliters iodine bound and end point potential. The end point of a titration is calculated from the observed potentials by the method of second differentials (7), eliminating the necessity for plotting the data to obtain this value. Using this method a value is obtained which is approximately 1.1% (amylose) higher than that given by the graphical method. This 1.1% difference represents the average found for 12 determinations on different samples

in which the per cent amylose was calculated by both methods. Since the maximum deviation noted between per cent amylose values calculated by the two methods was only 0.4% amylose when the correction factor of −1.1% was used with the end point method, the end point procedure appeared to be sufficiently accurate to justify its use in calculating the amylose content of the remainder of the starches

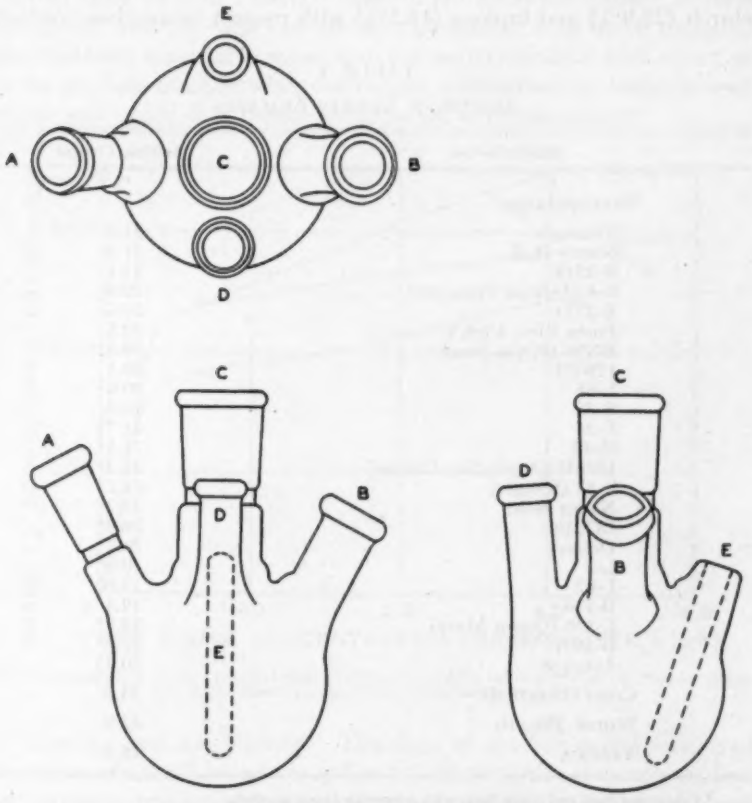


FIG. 3. Closed glass potentiometric iodine titration chamber.

selected for study. A considerable amount of time was thus saved without undue reduction in accuracy. It would appear that this rapid procedure is well suited for routine determinations in variety studies of the type under discussion. Any sample which is unusual with respect to amylose content may be retitrated and the per cent amylose calculated by the more accurate graphical method.

Discussion of Results

Amylose Content of Starch. Table I summarizes the results on 22 sweetpotato starches analyzed for amylose content, and also gives data obtained for corn starch, tapioca, and white potato starch. The per cent amylose in the sweetpotato starches varies from 17.5 to 21.7% but the great majority of the values lie between 20.0 and 21.7%. This places sweetpotato starch (average 20.4%) between white potato starch (22.9%) and tapioca (18.5%) with respect to amylose content.

TABLE I

RESULTS OF VARIETY ANALYSES

Starch Sample	Amylose Content
	%
SWEETPOTATO:	
Triumph	21.3
Nancy Hall	21.3
B-2518	21.1
L-5 (Pelican Processor)	20.8
B-2774	20.6
Porto Rico, Unit 1	20.5
B-196 (White Star)	20.4
129654	20.1
L-81	20.0
B-38	18.2
E-24	21.7*
K-40	21.5*
129655 (Australian Canner)	21.3*
L-12 (Ranger)	21.2*
Nancy Gold	21.2*
132056	20.9*
Dessert	20.1*
L-2	20.0*
L-82	19.6*
B-716	19.4*
L-126 (Queen Mary)	18.9*
B-4004	17.5*
Average	20.35
CORN (Defatted)	23.8
WHITE POTATO	22.9
TAPIOCA	18.5

* Calculated from end point data, with correction factor applied.

Corn starch is somewhat higher in amylose content than the root and tuber starches. The value obtained for tapioca is in reasonably good agreement with that reported by Schoch (10). Bates, French and Rundle (1) reported the amylose content of white potato starch to be 22%. More recently de Willigen and de Groot (14) reported the average amylose content of starches from 15 varieties of potatoes to be 23 to 28%; and Halsall and his associates (2) reported the amylose

content of starch in the growing potato tuber to vary from 15 to 19%. The extraction of sweetpotato starches and corn starch with methanol is described in the section on preparation of starches. The average of the amylose contents of the sweetpotato starches before extraction was 19.8%; the loss in weight during extraction was 0.104%; and the amylose content of the extracted starch was 20.4%. Corn starch lost 0.627% in weight during extraction; and the amylose content increased from 17.7 to 23.8%.[5] Since fatty acids are known to form complexes with the amylose fraction of starch, it is to be expected that the fatty material in whole starches would associate with a portion of the amylose fraction when the sample is dispersed for titration with iodine and would thus reduce its apparent amylose content.

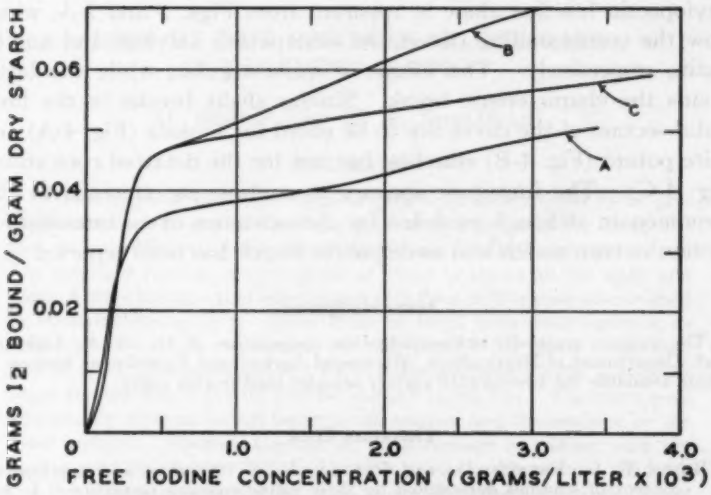


FIG. 4. The relation between the free iodine concentration and the iodine bound by (A) Tapioca starch, (B) White potato starch, and (C) Corn starch (defatted).

Amylose and Amylopectin. The slope of the horizontal section of the curve obtained for grams iodine bound per gram of starch vs. free iodine concentration in the case of tapioca starch (Fig. 4-A) was almost identical with that for sweetpotato starch (Fig. 2-B). This would indicate that the sweetpotato and tapioca amylopectin molecules take up iodine to the same extent and thus may be presumed to have similar structures, particularly with respect to the average lengths of their external branches. The corresponding section of the curve for white potato starch (Fig. 4-B) had a slightly steeper slope while that

[5] Even this latter value is lower than those commonly reported for corn starches. No explanation is available for the low value of the particular sample used.

for corn starch (Fig. 4-C) was considerably more horizontal. It can be inferred that white potato amylopectin has somewhat longer external branches, while corn amylopectin has relatively short external branches. Defatting of sweetpotato starch had no effect on the slope of its curve.

The presence of a slight break in the horizontal section of the curve for sweetpotato starch (Fig. 2-B) could be attributed to the presence of small amounts of a fraction intermediate between amylose and amylopectin in degree of branching. However, it is equally reasonable to assume that this break is due to the presence in the amylopectin fraction of a considerable number of external branches sufficiently long to bind considerable quantities of iodine at higher free iodine concentrations. That this second break in the curve is due to the amylopectin fraction alone is apparent from Figs. 1 and 2-A, which show the corresponding curves for sweetpotato amylose and amylopectin, respectively. The former is quite regular, while the latter retains the characteristic break. Similar slight breaks in the horizontal section of the curve are to be noted for tapioca (Fig. 4-A) and white potato (Fig. 4-B) starches, but not for the defatted corn starch (Fig. 4-C). The literature appears to contain no reference to this phenomenon, although evidence for the existence of an intermediate fraction in corn starch and sweetpotato starch has been reported (5).

Acknowledgment

The authors gratefully acknowledge the cooperation of Mr. W. S. Anderson, Head, Department of Horticulture, Mississippi Agricultural Experiment Station, in making available the sweetpotato variety samples used in this study.

Literature Cited

1. Bates, F. L., French, D., and Rundle, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. 65: 142–148 (1943).
2. Halsall, T. G., Hirst, E. L., Jones, J. K. N., and Sansome, F. W. The amylose content of starch present in the growing potato tuber. Biochem. J. 43: 70–72 (1948).
3. Hilbert, G. E. Private communication.
4. Hilbert, G. E., and MacMasters, M. M. Pea starch, a starch of high amylose content. J. Biol. Chem. 162: 229–238 (1948).
5. Hodge, J. E., Montgomery, Edna M., and Hilbert, G. E. Hydrolysis of the amylopectins from various starches with beta-amylase. Cereal Chem. 25: 19–30 (1948).
6. Kerr, R. W. E. Chemistry and Industry of Starch, 2nd Ed., p. 196. Academic: New York (1950).
7. Kolthoff, I. M., and Furman, N. H. Potentiometric Titrations, 2nd Ed., p. 95. Wiley, New York (1931).
8. Lansky, Sylvia, Kooi, Mary, and Schoch, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. 71: 4066–4075 (1949).
9. Schoch, T. J. Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. 64: 2957–2961 (1942).

10. SCHOCH, T. J. The fractionation of starch, in Advances in Carbohydrate Chemistry (ed. by W. W. Pigman and M. L. Wolfrom), Vol. 1, pp. 247–277. Academic: New York (1945).
11. SCHOCH, T. J., and JENSEN, C. C. A simplified alkali-lability determination for starch products. Ind. Eng. Chem. (Anal. Ed.) 12: 531–532 (1940).
12. WHISTLER, R. L., and HILBERT, G. E. Mechanical properties of films from amylose, amylopectin, and whole starch triacetates. Ind. Eng. Chem. 36: 796–798 (1944).
13. WHISTLER, R. L., and HILBERT, G. E. Separation of amylose and amylopectin by certain nitroparaffins. J. Am. Chem. Soc. 67: 1161–1165 (1945).
14. WILLIGEN, A. H. A. DE, and GROOT, P. W. DE. The relation between amylose and amylopectin in potato starch as a property of potato variety. Korte Ber. Proefsta. Aardappelverwerk., No. 7, 2 pp. (June 1947); Chem. Abstracts 43: 2005 (1949).
15. WILSON, E. J., JR., SCHOCH, T. J., and HUDSON, C. S. The action of *macerans* amylase on the fractions from starch. J. Am. Chem. Soc. 65: 1380–1383 (1943).

LOSS OF DDT RESIDUES IN BOX CARS CARRYING FLOUR[1]

B. BERCK[2] AND B. N. SMALLMAN[3]

ABSTRACT

Three types of DDT formulations (5% w:v) were applied to fir plywood surfaces, and one of these was applied to paper used for lining box cars, to give deposits of approximately 125 mg. of DDT per square foot. The resultant residues were exposed at three locations on the walls and floors of three box cars that were loaded with flour at Winnipeg and shipped to Montreal during July. Mean losses of DDT from wood surfaces, as determined by a spectrochemical method, were: DDT water suspension, 38.4%; DDT oil solution, 23.1%; DDT water emulsion, 20.7%. Car-lining paper treated with DDT oil solution showed 10.4% loss. The losses were significantly different except between the solution and the emulsion on the wood surfaces. Residues exposed on wood surfaces in contact with the load showed losses about 10% higher than similar residues above the load, and this difference is attributed to abrasion. Dislodgement by shock and vibration, and thermal degradation resulting from temperatures as high as 109°F., are postulated to account for the relatively high losses of DDT above the load. Car-lining paper treated with DDT oil solution showed superior retention of the insecticide at all locations and offers a simple means of decontaminating flour cars of DDT residues on arrival at destination.

Railway box cars may harbor insect pests of cereal products. The milling industry has recognized the necessity of protecting such products against infestation during transit (3, 4). Treatment of the interior surfaces of box cars with DDT has been advocated for this purpose (4). This proposal has raised two questions: Will DDT

[1] Manuscript received November 13, 1950. Contribution No. 2725, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.
[2] Technical Officer, Stored Product Insect Laboratory, Winnipeg, Manitoba.
[3] Officer-in-Charge, Stored Product Insect Laboratory, Winnipeg, Manitoba.

deposited on the car surfaces remain unchanged and available for the protection of the cargo during long hauls? And will sufficient DDT be retained on the car surfaces to constitute a contamination hazard to subsequent cargoes such as bulk grain? These questions prompted the present study.

In Canada, flour may be shipped by rail for distances up to 2,300 miles, and may be in transit as long as 12 days. During this period, DDT deposited on the floors and walls of box cars will be subjected to abrasion by the load, to shock and vibration, and possibly to high temperature and humidity. These factors would be expected to alter the original deposit by dislodging part of it or by promoting chemical degradation. In either case, any change in the insecticidal deposit may be modified by its location in the car and the type of formulation in which the DDT is applied.

This study shows the losses of DDT that occurred when DDT in oil solution, in water emulsion, and in water suspension were applied at three locations in each of three box cars loaded with flour at Winnipeg, Manitoba, and shipped to Montreal, Quebec. Since flour cars are normally lined with paper, the loss from DDT-impregnated paper was determined under the same conditions.

Materials and Methods

Fir plywood boards and car-lining paper were sprayed with known amounts of 5% DDT in oil solution, water suspension, and water emulsion. The sprayed surfaces were attached at three different locations to the interior surfaces of three box cars. The cars were then loaded with flour in the normal way and shipped from Winnipeg to Montreal during July. During transit, temperatures were recorded at the three locations in one car. After the unloading at Montreal, the test surfaces were removed and returned to Winnipeg. The amounts of DDT remaining on the test surfaces were determined by the Schechter-Haller spectrochemical method (7); and, by comparison with the corresponding control surfaces, the losses of DDT during transit were calculated.

Board and paper surfaces were constructed to simulate those in Class A cars used for flour shipments. The board surfaces were of select 3-ply fir plywood, all from the same stock. The plywood was machine-sawn into 10 × 10 in. squares. Each board was ruled into four 3 × 3 in. squares, as shown in Fig. 1. The paper surfaces consisted of squares of kraft car-lining paper stapled to 10 × 10 in. squares of fir plywood and marked off in squares in the same way as the board surfaces.

The DDT formulations were applied to these surfaces by spraying them in a laboratory spray tower under conditions designed to give a deposit of approximately 125 mg. of DDT per square foot of surface. The accuracy with which such a deposit could be applied was first determined by spraying glass plates, 3 × 3 in., laid out as quadrants of a 6 × 6 in. square in the same manner as the test surfaces shown in Fig. 1. Three groups of 20 glass plates were each sprayed with one of the following: DDT oil solution, DDT water suspension, and DDT water emulsion.[4] The mean deposits and standard deviations ob-

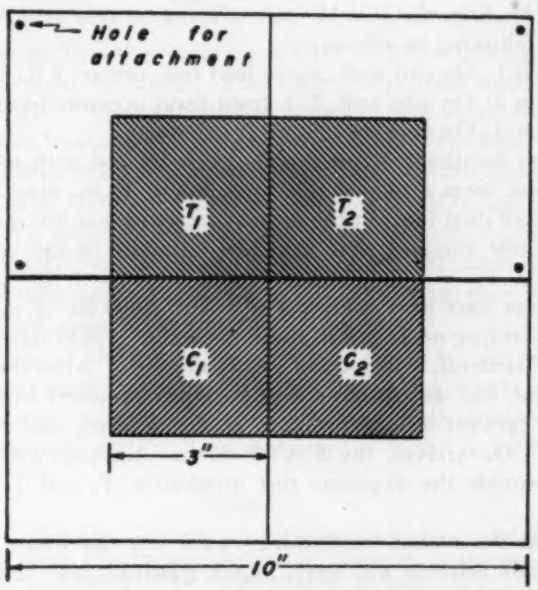


FIG. 1. Arrangement of surfaces for application of DDT deposit. Subsequently, the upper half, containing test areas T1 and T2, was exposed in the box car; the lower half, containing control areas C1 and C2, was retained at the laboratory. The unhatched area permitted handling without disturbing the deposit on the test and control areas.

tained for each group were: 139.8±3.1, 124.4±2.7, and 121.4±8.2 mg. of DDT per square foot, respectively. Direct evaluation of the amounts of DDT deposited on the board surfaces was impossible because of absorption by the wood (1); but the test surfaces were sprayed under the same conditions as the glass plates and were assumed to have received equal deposits. Paper surfaces were sprayed with the DDT oil solution only.

[4] An oil solution of technical grade DDT (dichlorodiphenyltrichloroethane) in No. 9 refined kerosene with 15% xylene as intermediate solvent; a suspension made from C.I.L. 50% wettable DDT; an emulsion made from Invarco 25% DDT emulsion concentrate.

Each board was then machine-sawn in half to provide a section for exposure in the cars and a corresponding section to be held in the laboratory as a control. The first control area (C_1) was analyzed 24 hours after spraying, and the second (C_2) was analyzed 45 days later, when the test surfaces were returned from Montreal. During this 45-day interval, efflorescence of absorbed DDT occurred (1), invalidating the use of C_1 as a duplicate control area. Accordingly, C_2 was used to determine the loss of DDT from the corresponding test areas during exposure in the cars. The control areas were held in the laboratory at room temperatures, and protected from light and dust.

Before loading, the 5 × 10 in. test surfaces were attached to each car in the following locations:

Location 1: On end wall, above load line, center, 7 ft. from floor.

Location 2: On side wall, 2 ft. from floor, opposite location 3.

Location 3: On floor, center, 10 ft. from end.

At these locations, 3 test boards, each treated with one of the 3 formulations, were attached side-by-side and ½ in. apart. The car interiors were then lined in the normal way with car-lining paper, and the paper test surfaces were attached adjacent to the covered test boards.

The three cars were each loaded with 60,000 lb. of sacked flour, and left Winnipeg on July 8 in the same train. Eight days later they arrived at Montreal, a distance of 1,415 rail miles. After the unloading at Montreal, the test surfaces were carefully removed from the cars, packed to prevent any disturbance of the residues, and returned to Winnipeg. On arrival, the 5 × 10 in. test surfaces were machine-sawn to provide the duplicate test quadrants, T_1 and T_2, shown in Fig. 1.

To strip the surface residue from each test quadrant, 100 ml. of reagent grade benzene was used. Each quadrant was first placed at an angle of 45° in a funnel 7 in. in diameter and fitted with a tight plug of glass wool to retain benzene-insoluble matter. A stream of benzene was directed at an angle of 90° to the surface from a distance of 6 in. The benzene was dispensed from an all-glass wash bottle pressurized with air at 5–10 mm. Hg line pressure. During the stripping operation, the test surface was quarter-turned 4 times to rotate the grain of the wood. After approximately 85 ml. was delivered, about 15 ml. of benzene was used to wash the funnel. Untreated board surfaces were similarly stripped to provide "blanks."

The paper surfaces were stripped by folding the 3 × 3 in. quadrants and immersing these in 100 ml. of benzene. The amount of DDT recovered in this way was found to be the same as when the paper was stripped by the benzene "stream" method used with the boards.

A record of the temperatures in one of the cars during transit was obtained. Three Friez recording thermographs were mounted on platforms of 1 in. wood cushioned with sponge rubber and housed in perforated wooden boxes. The boxes were placed in one of the cars, alongside the three locations assigned to the test surfaces. To minimize the shock of shunting, the thermographs were placed so that the arms of the recording pens were parallel with the long axis of the car. The sacked flour was loaded directly from the mill packers and piled carefully around the boxed instruments.

Results

Table I shows that the retention of DDT on paper was significantly greater than that on wood. The mean loss from wood surfaces treated with DDT oil solution, for instance, was more than twice that from paper treated with the same formulation. On wood surfaces, the type of formulation had a significant effect on the loss of DDT. The mean loss of DDT from boards treated with DDT water suspension was 38.4% and was significantly higher than that from boards treated with DDT oil solution (23.1%) and DDT water emulsion (20.7%). Smaller losses occurred above the load than below it, regardless of substrate or formulation.

Table II shows the mean temperatures, based on 2-hr. intervals, obtained at the three locations in the box car during 8 days in transit. The mean temperature recorded in the flour car over the 8-day period was approximately 90°F. Temperatures as high as 109°F. were

TABLE I

MEAN LOSSES (%) OF DDT FROM TREATED SURFACES IN THREE BOX CARS LOADED WITH FLOUR AT WINNIPEG, MANITOBA, AND SHIPPED TO MONTREAL, QUEBEC

Treatments	Locations in Cars			Means for Treatments[1]
	Above Load	Side Wall	Floor	
DDT water suspension on wood	32.4	39.5	43.2	38.4
DDT oil solution on wood	16.7	24.4	28.2	23.1
DDT water emulsion on wood	13.7	25.5	22.9	20.7
DDT oil solution on paper	5.1	10.9	15.3	10.4
Means for locations[2]	16.9	25.1	27.4	

[1] Least significant difference, 7.6.
[2] Least significant difference, 6.8.

TABLE II

MEAN TEMPERATURES (°F.) AT THREE LOCATIONS IN ONE BOX CAR LOADED WITH FLOUR AT WINNIPEG, MANITOBA, AND SHIPPED TO MONTREAL, QUEBEC, JULY 7–14

Location	Days in Transit								Mean
	1	2	3	4	5	6	7	8	
Above load	87.5	95.0	90.5	86.0	86.5	95.5	—[1]	—[1]	90.0
Side wall	93.5	93.0	92.5	86.0	86.5	90.0	81.0	84.0	88.3
Floor	92.0	96.0	96.0	92.5	89.0	90.0	88.0	85.0	91.1

[1] Record indistinguishable.

recorded above the load. The flour, which was loaded directly from the packers to the car, showed temperatures of 99–101°F. when loaded. High outside temperatures that at times exceeded 100°F. must have helped to maintain the relatively high temperatures shown in Table II.

Discussion

The results obtained with wood surfaces indicate that DDT residues on the surfaces of box cars carrying flour will suffer considerable losses during a long haul. The amount lost appears to be influenced by the type of formulation in which the DDT is applied and by the location of the DDT residue in the cars. For example, between formulations, wood surfaces treated with DDT suspension showed the largest losses; and, within formulations, residues exposed in contact with the load showed larger losses than those above the load. It follows that the agencies promoting loss of DDT were more effective against the suspension than against the other two formulations, and more effective below the load than above it.

Loss of DDT residues in the cars must have resulted from mechanical removal, or chemical decomposition, or both. Mechanical removal of DDT residues may have been affected by shock and vibration during transit, and by abrasion between the test surfaces and the load. Partial decomposition of DDT may have resulted from exposure to the moderately high temperatures prevailing in the cars (5).

Since residues exposed above the load were free from abrasion, the observed losses may be attributed to removal by shock and vibration, or thermal decomposition, or both. In any event, the factors promoting the loss were more effective against the residue deposited by the suspension than against those of the other two formulations. There appears to be no information indicating that thermal decomposition of DDT residues is influenced by the type of

formulation applied. The loss resulting from decomposition could not have exceeded the 5.1% loss from paper surfaces above the load and may have been much less, since part of the 5.1% was probably removed mechanically. Therefore, decomposition of DDT seems to have been a minor factor. More probably, as is often supposed, the light, powdery residues deposited by suspensions of water-wettable powders are dislodged more easily than those deposited by solutions or emulsions. However, there is no clear evidence on this point (4).

Abrasion between the test surfaces and the load would be expected to dislodge some of the residue and result in higher losses below the load than above it, as was in fact observed. The mean loss of DDT on wood surfaces in contact with the load was about 10% higher than the loss above the load. This difference may reasonably be attributed to removal of residues by abrasion.

Car-lining paper treated with DDT oil solution retained significantly more of the insecticide than wood surfaces treated with any of the three formulations. The factors promoting loss of DDT above the load were relatively ineffective against residues on the paper, and the effect of abrasion by contact with the load was reduced. During transit the mean loss at all locations was 10.4%, or half the smallest loss observed for wood surfaces.

The superior retention of DDT on car-lining papers appears to offer a simple method for holding a maximum amount of the insecticide in position to protect the load during transit and for decontaminating the cars on arrival at destination. Flour cars are normally lined with kraft paper before loading, and Cotton, Wagner, and Winburn (3) found that the lining acts as a temporary barrier to resident insects during short hauls. The use of DDT-impregnated paper would be expected to augment and extend the effectiveness of this mechanical barrier. Monro *et al.* (6) have shown that wallpaper, treated with DDT at approximately the same rate as was the car-lining paper in this study, was highly toxic to houseflies and retained this toxicity for at least a year. Car-lining paper could be impregnated with DDT most economically at the paper mill, and the increased cost of the paper should be considerably less than the cost of spraying cars individually at the flour mill. Additional protection could be obtained by covering the top surface of the load also, thus completing a box envelope of treated paper around the load. On arrival at destination, removal of the car-lining paper would effectively decontaminate the car of DDT residues.

The results indicate that car surfaces directly sprayed with DDT retain 60 to 80% of the original deposit after unloading. This residue

constitutes a potential source of contamination for subsequent loads such as bulk grain. A simple calculation shows that if all this residue was removed by a subsequent load of wheat, the wheat would contain about 1.5 p.p.m. of DDT. However, this low value supposes complete mixing of the peripheral grain with the main bulk. The recent demonstration (2) that DDT may be transferred, partly in the vapor phase, from impregnated cotton sacks to the enclosed flour suggests the possibility that the flour load itself may become contaminated during transit in cars sprayed with DDT or lined with DDT-impregnated paper.

Acknowledgment

The authors wish to acknowledge the assistance of H. A. U. Monro, Officer-in-Charge, Fumigation Laboratory, Division of Plant Protection, Canada Department of Agriculture, Montreal, who supervised the removal and packing of the test surfaces at Montreal.

Literature Cited

1. Berck, B., and Smallman, B. N. Absorption and efflorescence of DDT deposited on wood. Soap and Sanit. Chem. (In press).
2. Butterfield, D. E., Parkin, E. A., and Gale, M. M. The transfer of DDT to foodstuffs from impregnated sacking. J. Soc. Chem. Ind. **68**: 310–313 (1949).
3. Cotton, R. T., Wagner, G. B., and Winburn, T. F. Infestation in transit. Am. Miller **68**, No. 4, 38–40, 60, 62 (1940).
4. Durham, R. K. A guide for flour mill sanitation through modern methods for prevention of insects and rodents in flour mills. Millers' National Federation. Chicago (1949).
5. Gunther, F. A., Lindgren, D. L., Elliot, M. I., and LaDue, J. P. Persistence of certain DDT deposits under field conditions. J. Econ. Ent. **39**: 624–627 (1946).
6. Monro, H. A. U., Beaulieu, A. A., and Delisle, R. DDT residues: Their toxicity to houseflies on various surfaces and materials. Soap and Sanit. Chem. **23**: No. 8, 123, 125, 127, 129, 143, 145 (1947).
7. Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L. Colorimetric determination of DDT. Color test for related compounds. Ind. Eng. Chem. Anal. Ed. **17**: 704–709 (1945).

DENATURATION OF SOYBEAN PROTEIN WITH ALCOHOLS AND WITH ACETONE[1]

A. K. SMITH, VERNON L. JOHNSEN,[2] and ROBERT E. DERGES

ABSTRACT

The denaturation of the protein in soybean meal by various concentrations of methanol, ethanol, isopropanol, and acetone has been investigated in the temperature range of 30° to 75°C. and for various time intervals. Denaturation was measured by the change in water dispersibility of the protein before and after treatment of the meal with the denaturing systems. Judged by this test, water is less effective as a denaturant under certain conditions than the pure organic solvents. The alcohol-water solutions, at 40 to 60% concentration, are the most effective. The denaturation reaction is nearly complete in 5 minutes.

In the production from soybeans of a proteinaceous product called Gelsoy (1), undenatured oil-free flakes (hexane-extracted) are washed with ethanol at about 30° to 40°C. The alcohol is evaporated from the flakes, which are then extracted with water, and the water solubles recovered by spray drying. It has been found that removal of the alcohol is a critical step in the process and that success in Gelsoy production depends largely on removing the alcohol without appreciable denaturation of the protein. Preliminary to designing equipment for alcohol desolventizing, a study was made of the denaturing action of ethanol and mixtures of ethanol and water.

As methanol, isopropanol, and acetone are frequently used for precipitation, dehydration, and extraction of proteins and biological materials containing proteins, they were included in this study. The extent of protein denaturation was determined as the difference in the amount of water dispersible nitrogen in the meal before and after treatment with alcohol or acetone solutions.

Organic solvent denaturation has been observed by many investigators (5) and it has been recognized that the denaturing response is dependent upon the specific protein as well as the denaturing agent. Mann and Briggs (3) investigated, by electrophoretic methods, the proteins occurring in the water extract of soybean meal, before and after heating the dispersion and in the water extract of meal which had been washed with alcohol. They found that treatment of soybean meal with hot methanol and ethanol decreased the water dis-

[1] Manuscript received December 7, 1950. Contribution from the Northern Regional Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] This paper is based on work submitted by Vernon L. Johnsen in partial fulfillment of the requirements for the M.S. degree at Bradley University, Peoria, Illinois.

persibility of the protein, and that dispersibility of the fraction designated by Osborne (6) as glycinin was the most affected. Of particular interest is their observation that while hot alcohol treatment renders the globulin fraction almost completely non-dispersible in water, heating the proteins in an aqueous dispersion insolubilizes primarily the other protein components; also, that the effect of heat is dependent upon the amount of water present, an observation which agrees with that of other investigators.

Of further interest is Mann and Brigg's observation that in mixed protein systems, such as occur in soybean meal, the denaturation caused by heating a water dispersion results in interaction of the several proteins. They noted a similar reaction when their protein dispersions were allowed to stand at room temperature for a period of time. An analogous explanation might be postulated for alcohol denaturation of the soybean protein system; however, direct experimental evidence to support such a theory is yet to be supplied.

Materials and Methods

Fat-free soybean meal was prepared by extracting dehulled and flaked soybeans with hexane in a laboratory extractor. The solvent was removed by evaporation at room temperature, and the flakes ground in a Wiley mill equipped with a 0.5 mm. screen. One batch of soybean flakes was used for all experiments. The denaturation was carried out by two different procedures. In the first procedure the nitrogen dispersion experiments were made without removing the denaturing solvent from the meal; in the second or alternate procedure, the denaturing solvent was removed from the meal by evaporation at room temperature.

In the first procedure the treatment was carried out on samples of 1.25 g. of ground meal, which were weighed into small-mouth centrifuge bottles. The alcohol, water, or alcohol-water solution used in the denaturation studies was added in sufficient amount to wet the meal and have some excess. The meal absorbed the water-denaturant mixture in different proportions, depending on its composition, therefore the denaturing solution varied in amount from 6.25–15.00 ml. As an example, a treatment with 70% methanol was carried out by mixing 5.67 g. methanol and 2.43 g. water, adding this to the meal in a 250 ml. centrifuge bottle in constant temperature bath. A reflux condenser was attached to prevent loss of denaturant at the higher temperatures. After the desired time, the bottles were cooled rapidly in an ice bath to room temperature. The dispersible nitrogen was determined by the following procedure. Sufficient water was added to bring the total amount of solution to 150 ml. and the bottles shaken

mechanically for 30 minutes. After centrifugation, a 50-ml. aliquot of the supernatant solution was removed for Kjeldahl nitrogen analysis, and the dispersible nitrogen calculated on the basis of total nitrogen in the meal. All results are the average of two or more determinations.

Since the quantity of denaturant varied from 6.25–15.00 ml., a series of preliminary experiments were made with suitable variations of denaturing solution to the meal to test this effect. The tests showed that when there was an excess of denaturant over the amount required to wet the meal, the ratio of denaturant to meal made no measurable difference in the denaturation results.

Another possible source of error to be considered when using this procedure is the decrease in nitrogen solubility caused by the presence of the small amount of alcohol in the dispersing medium. The work of Nagel, Becker, and Milner (4) showed that the presence of 10% methanol lowered nitrogen dispersibility 4% while 10% ethanol lowered it 8.3% when a solvent to meal ratio of 40:1 was used. In the present investigation for the first procedure, the solvent to meal ratio was 120:1. Test runs showed that the small amount of alcohol in the solvent caused no determinable error. This is not in disagreement with Nagel, Becker, and Milner because the water-meal ratio in the present work was much greater than that used in their method.

In the second or alternate technique, the defatted flakes were treated with the alcoholic solutions at the desired temperature and time and then spread in a thin layer on the laboratory bench until they appeared dry. The dried flakes were ground and 2.5 g. were weighed into a centrifuge bottle, 100 ml. of water were added, and the bottles shaken for 0.5 hour. The suspension was clarified in a centrifuge, and a 20-ml. aliquot was removed for Kjeldahl nitrogen determination. This method was less satisfactory than the first, since more total time was consumed and additional denaturation occurred during the long and variable drying time after the alcohol treatment. The removal of the alcohol by evaporation exposes the meal to all alcohol concentrations between that of the original treatment and zero concentration and increases the denaturing effect for most alcohol concentrations. Furthermore, nitrogen and moisture determinations were required for each sample of alcohol-treated meal for quantitative determination of soluble nitrogen on a dry basis.

Results

The changes in nitrogen dispersion resulting from treatment with pure methanol, ethanol, isopropanol, and corresponding alcohol-water mixtures for 30 minutes in the range of 30°–75°C., and the acetone

TABLE I

CHANGES IN DISPERSIBILITY OF NITROGEN OF SOYBEAN MEAL IN WATER AFTER TREATMENT OF THE MEAL WITH VARIOUS ALCOHOL-WATER AND ACETONE-WATER MIXTURES

Denaturing Medium	pH of Dispersion	Total nitrogen dispersed in water after 30 minute treatment with alcohol-water and acetone-water solutions; alcohol or acetone—concentration, % by weight												
		0	5	10	20	30	40	50	60	70	80	90	95	100
		%	%	%	%	%	%	%	%	%	%	%	%	%
Alcohol-Water at 30°C.[1]														
Methanol	6.5	90.4	90.4	88.2	78.9	67.7	51.9	36.4	30.0	33.5	41.2	58.4	60.9	61.4
Ethanol	6.5	90.4	90.1	86.5	72.5	42.3	30.2	31.5	35.9	58.0	62.2	66.0	72.5	76.7
Isopropanol	6.5	90.4	89.1	85.3	51.6	29.5	27.4	36.1	56.3	62.6	65.9	75.3	78.4	80.8
Alcohol-Water at 75°C.[1]														
Ethanol	6.5	79.5	48.2	27.1	18.2	15.8	13.9	11.0	9.7	9.5	9.6	17.4	32.5	64.6
Isopropanol	6.5	79.5	79.4	48.0	26.5	25.5	17.6	12.9	8.8	8.1	11.5	56.2	60.7	67.1
Alcohol-Water at 60°C.[1]														
Methanol	6.5	88.8	88.4	72.0	37.3	16.6	11.7	10.3	9.1	10.0	11.9	16.4	22.3	29.4
Acetone-Water at 30°C.[1]														
Acetone	6.5	90.4	90.4	86.0	73.8	61.4	61.6	62.4	63.6	66.4	67.1	74.7	79.8	81.0
Ethanol-Water at 75°C.[2]														
Ethanol-Water	6.5	39.3	26.6	—	14.1	13.6	11.9	10.2	9.0	8.9	11.6	—	47.4	63.4
Ethanol-Water	9.3	51.8	35.2	—	—	23.5	21.7	19.6	19.9	23.3	48.4	—	74.7	79.1

[1] Alcohol-water treatment and nitrogen dispersibility by first procedure. See text.
[2] Alcohol-water treatment by second procedure.

system at 30°C. (all concentrations on a weight basis) are shown in Table I.

At alcohol concentrations of about 50% by weight and above and at a temperature of 30°C., the denaturing effect of the alcohol system increases in the order of isopropanol, ethanol, and methanol. At alcohol concentrations below 50% the order of denaturation is in the reverse order. At 75°C. the denaturing effect of ethanol is somewhat

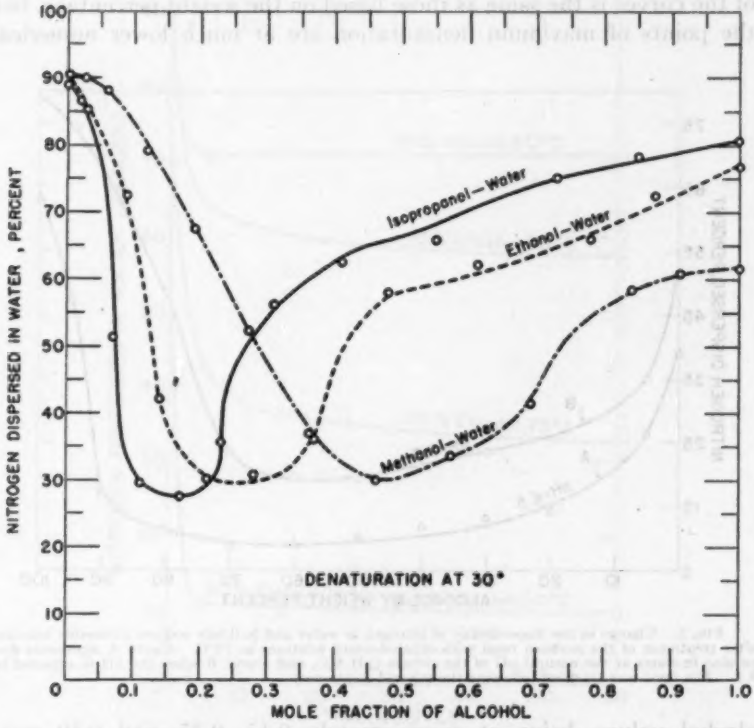


FIG. 1. Change in dispersibility of nitrogen in soybean meal in water (pH 6.5) after treatment of the meal with various alcohol-water mixtures at 30°C.

greater than for isopropanol except in the range of 60–70% alcohol where the values are reversed. While the extent of the reversal is small, repeated determinations of these values convinced us that the reversal is real. The soluble nitrogen occurring at maximum denaturation (75°C. and 60–70% alcohol) is partly non-protein nitrogen and partly protein. Soybean meal contains 5% or more of non-protein nitrogen, based on the total nitrogen in the meal, and the

difference between this approximate non-protein value and the minimum in the curve represents protein not insolubilized by the alcohol treatment.

The acetone-water systems have the same denaturing patterns as the alcohol-water systems but the extent of denaturation of the acetone-water is less.

Denaturation at 30°C. for the three alcohol systems, when calculated on a mole fraction basis, is shown in Fig. 1. The general shape of the curves is the same as those based on the weight percentage, but the points of maximum denaturation are at much lower numerical

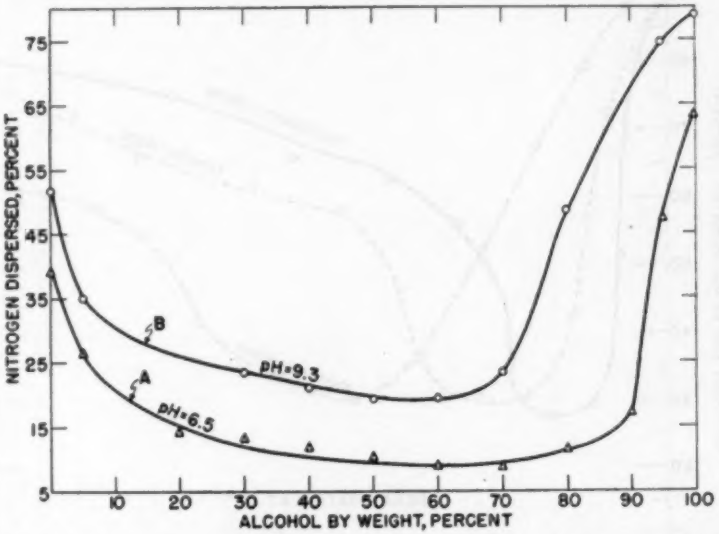


FIG. 2. Change in the dispersibility of nitrogen in water and in dilute sodium hydroxide solution after treatment of the soybean meal with ethanol-water mixtures at 75°C. Curve A represents dispersion in water at the natural pH of the system (pH 6.5), and Curve B when the pH is adjusted to 9.3. The meal was air dried following the ethanol treatment.

alcohol values, being at approximately 0.17, 0.27, and 0.50 mole fractions of isopropanol, ethanol, and methanol, respectively.

Because our soybean meal contained about 7% moisture, it is evident that the concentration of alcohol would change slightly after its addition to the meal; consequently the 100% alcohol points on the curves represent slightly lower concentrations. The effect of this small amount of water was estimated by using absolute ethanol on soybean meal brought to a very low moisture content by placing the meal for 1 week in a desiccator over phosphorous pentoxide. The denaturing effect of the absolute ethanol on the dehydrated meal was

essentially the same as on meal containing its normal moisture content, indicating that no error was introduced by the normal moisture in the flakes.

In Fig. 2 are dispersion curves of soybean meal protein that was denatured for 30 minutes at 75°C. according to the alternate procedure in which the alcohol-water was removed by atmospheric evaporation. The results show that the alternate procedure lowers the dispersibility

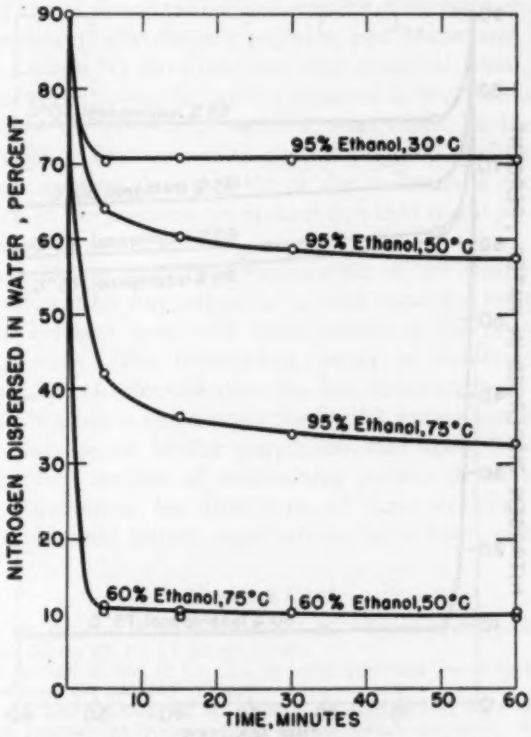


FIG. 3. Change in dispersibility of nitrogen of soybean meal in water with time of treatment of the meal with various concentrations of ethanol.

of the protein more than the first method. This difference is explained by the long time required for evaporation of the alcohol-water from the flakes and by the change in concentration of alcohol in contact with the flakes. In the first method at 75°C., pure water has less denaturing effect than the pure alcohols, whereas in the alternate method pure water has a much greater denaturing effect. These experiments indicate that wetting of the meal with water and subsequent drying in

the atmosphere has a greater effect on dispersibility of the protein than heating the wet meal at 75°C. for 30 minutes.

The effect of time and temperature on denaturation at various alcohol concentrations is shown in Figs. 3 and 4. In general, the data show that denaturation proceeds very rapidly for the first few minutes and slowly thereafter; in fact, regardless of the extent of denaturation, the major portion of the reaction is completed in less than 5 minutes.

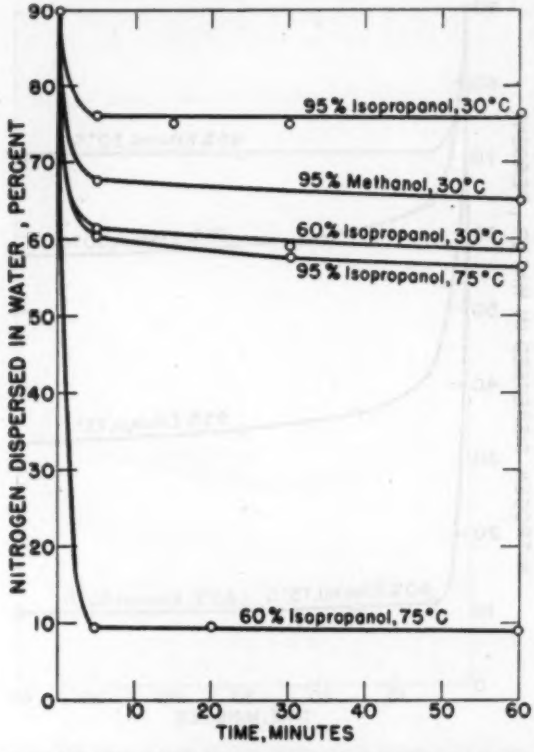


FIG. 4. Change in the dispersibility of the nitrogen of soybean meal in water with time of treatment of the meal with various concentrations of isopropanol and with 95% methanol.

At constant temperature and for a given alcohol concentration, the time curves are of the same order as shown by the concentration data in Table I.

Discussion

The effect of different concentrations of alcohols and of acetone on the denaturation of the proteins in soybean meal cannot be predicted

from the denaturing effects of the individual components of the solutions.

Denaturation proceeds rapidly at room or higher temperatures for all alcohol concentrations between 10 and 90% and the reaction appears to be almost complete in 5 minutes.

The data are not especially suitable for theoretical speculation or for correlation with fundamental theories on protein denaturation because of the heterogenous character of the protein system. Briggs and Mann (1) have shown that water extracts of soybean meal contain seven electrophoretically distinct proteins, and Mann and Briggs (3) and Spiegel-Adolph (7) have evidence that extent of denaturation by alcohol varies with the specific protein involved in the reaction. Thus, denaturation of the protein in soybean meal would be the result of several reactions. The work of Mann and Briggs (3) indicates that in mixed protein systems denaturation of the molecule is accompanied by interaction of the proteins, an explanation that might be applicable to our systems.

Regardless of the theoretical explanation of the results reported, they are significant for any industrial process requiring the removal of alcohol from soybean meal with maintenance of the protein in the undenatured state. The dominating factor in desolventizing the flakes is time, the shorter the time the less denaturation. However, alcohol concentration is also a major factor if it ranges between 10 and 90%. The data point to the suggestion that flash desolventizing would be the best method of maintaining protein denaturation at a minimum. Equipment for this type of desolventizing has been designed, tested, and patent applications have been made for the process.

Literature Cited

1. Beckel, A. C., Belter, P. A., and Smith, A. K. A new protein product, Gelsoy. Soybean Digest **10**: (1) 17–18, 40 (1949).
2. Briggs, D. R., and Mann, R. L. An electrophoretic analysis of soybean protein. Cereal Chem. **27**: 243–257 (1950).
3. Mann, R. L., and Briggs, D. R. Effects of solvent and heat treatments on soybean proteins as evidenced by electrophoretic analysis. Cereal Chem. **27**: 258–269 (1950).
4. Nagel, R. H., Becker, H. C., and Milner, R. T. The solubility of some constituents of soybean meal in alcohol-water solutions. Cereal Chem. **15**: 766–774 (1938).
5. Neurath, H., Greenstein, J. P., Putnam, F. W., and Erickson, J. O. The chemistry of protein denaturation. Chem. Rev. **34**: 157–265 (1944).
6. Osborne, T. B., and Campbell, G. F. Proteids of the soy bean (*Glycine hispida*). J. Am. Chem. Soc. **20**: 419–428 (1898).
7. Spiegel-Adolf, Mona S. Neue Berträge zur Frage der Eiweissdenaturierung. I. Alkoholdenaturierung. Biochem. Z. **204**: 1–13 (1929).

PHYSIOLOGICAL EFFECTS OF DISTILLERS' DRIED GRAINS DERIVED FROM ACID-SACCHARIFIED CORN MASH[1]

ANTHONY M. AMBROSE

ABSTRACT

Albino rats were used to determine the physiological effects of increasing amounts of acid-saccharified corn mash, which contained 4.3% sodium sulfate and 15.3% calcium sulfate. For comparison rats were fed diets containing 0.5% or 1% sodium sulfate and 2% or 4% calcium sulfate singly and in combination for periods up to 200 days. Observations were made periodically of gross appearance, growth, food consumption, and consistency of fecal droppings, followed by gross examination of thoracic and abdominal viscera at necropsy, and histopathological study of the organs. From these studies it is concluded that sodium sulfate and calcium sulfate, arising from the neutralization of the sulfuric acid used in the saccharification of the ground corn mash, are toxic ingredients of the diet, producing a mild laxative action.

The increased demand for alcohol has resulted in a large increase in the production of residues of potential feed value. A number of types of fermentation residues are available, the nature of which differs with the starting materials and their treatment before and after fermentation.

Studies on the utilization and nutritive value of corn distillers' by-products, collectively referred to as distillers' dried grains and distillers' dried solubles, as supplements in animal feeds, have been summarized by Bauernfeind and Boruff (1) and reviewed by Synold, Carrick, Roberts, and Hauge (5). Fairbanks, Krider, and Carroll (3) and Krider, Fairbanks, and Carroll (4) have reported equally favorable results on the use of distillers' by-products in swine rations. Bauernfeind, Garey, Baumgarten, Stone, and Boruff (2) have reported data on the vitamin, mineral, and amino-acid content of corn distillers' by-products.

At present, the bulk of distillers' dried grains produced in this country is derived from grain mashes saccharified with dried malt. However, there are other saccharification processes known and practiced, such as the conversion with fungal amylases or by acid hydrolysis. The latter process is widely used in foreign countries and may be considered for use in the United States if a shortage of malting

[1] Manuscript received December 26, 1950. Contribution from the Pharmacology Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany 6, California.

barley should occur. In this process ground corn is hydrolyzed with sulfuric acid, and the excess acid neutralized with sodium hydroxide and calcium carbonate before fermentation with yeast. After fermentation has been concluded, the alcohol is stripped from the so-called beer and the resulting stillage is evaporated to a thick sirup. The sirup is drum dried to yield the feed which was used in the studies herein reported.

This residue differs from distillers' by-products mainly in the ash content because of the presence of large amounts of calcium and sodium sulfates resulting from the neutralization process. In other respects the residue obtained in the acid-saccharification processes is comparable to distillers' dried grains with solubles added obtained by the conventional malt saccharification. Studies were undertaken, therefore, to determine the effect on rat growth of diets containing increasing percentages of acid-saccharified corn mash. For comparison, rats were fed diets containing sodium sulfate or calcium sulfate, singly or combined.

Materials and Methods

The residues of acid-saccharified corn mashes (ASC) used in these studies had the following composition: moisture 8.5, ash 23.6, protein 25.9, petroleum ether extract 7.4, sodium sulfate 4.3, and calcium sulfate 15.3%; riboflavin 8.4, pantothenic acid 13.5, nicotinic acid 77.0, pyridoxine 3.8, and biotin 0.11 μg./gram.

TABLE I

INFLUENCE OF ADDITIONS OF ACID-SACCHARIFIED CORN MASH (ASC) AND OF SODIUM AND CALCIUM SULFATES ON THE GROWTH OF RATS
Time: Approximately 200 Days

Supplement	No. of Rats		Average Weight (grams)		
	At Start	At End	At Start	At End	Increase ±S.E.[1]
Control	5	5	42.6	225.2	182.6±7.8
2.5% ASC	5	5	42.8	209.6	166.8±4.5
5% ASC	5	5	42.0	189.8	147.8±4.7*
10% ASC	5	5	43.2	206.0	162.8±4.3*
15% ASC	5	5	41.8	194.0	152.2±4.3*
20% ASC	5	5	41.8	181.8	140.0±4.0*
25% ASC	5	5	41.6	170.2	128.6±2.1*
2% $CaSO_4$	5	5	42.6	211.4	168.8±9.9
4% $CaSO_4$	5	5	42.6	187.0	144.4±8.8*
0.5% Na_2SO_4	5	5	41.4	211.8	170.4±2.8
1% Na_2SO_4	5	5	41.4	212.2	170.8±3.2
2% $CaSO_4$+0.5% Na_2SO_4	5	4	41.2	194.7	153.7±3.5*
4% $CaSO_4$+1% Na_2SO_4	5	5	42.0	188.6	146.4±6.7*

[1] S.E. = Standard Error of the Mean.
* Significant difference, P (0.05). Difference between mean weight increase of rats on experimental diets as compared with control.

Two series of feeding tests were carried out; in one, increasing percentages of ASC were added to the control diet; in the other, two levels of sodium sulfate and calcium sulfate, both singly and combined, were added to the control diet. The experimental diets were prepared by thoroughly grinding the supplements in a mortar and mixing with the basic diet, which consisted of a commercial dog food.

Young weanling female white rats weighing 40 to 45 g. were divided into groups of five, caged together, and placed on the respective dietary supplements. The general design of the experiment is given in Table I. The rats had free access to food and water at all times. All rats were weighed once each week, and records were kept of body weight, food consumption, and general appearance. Observations

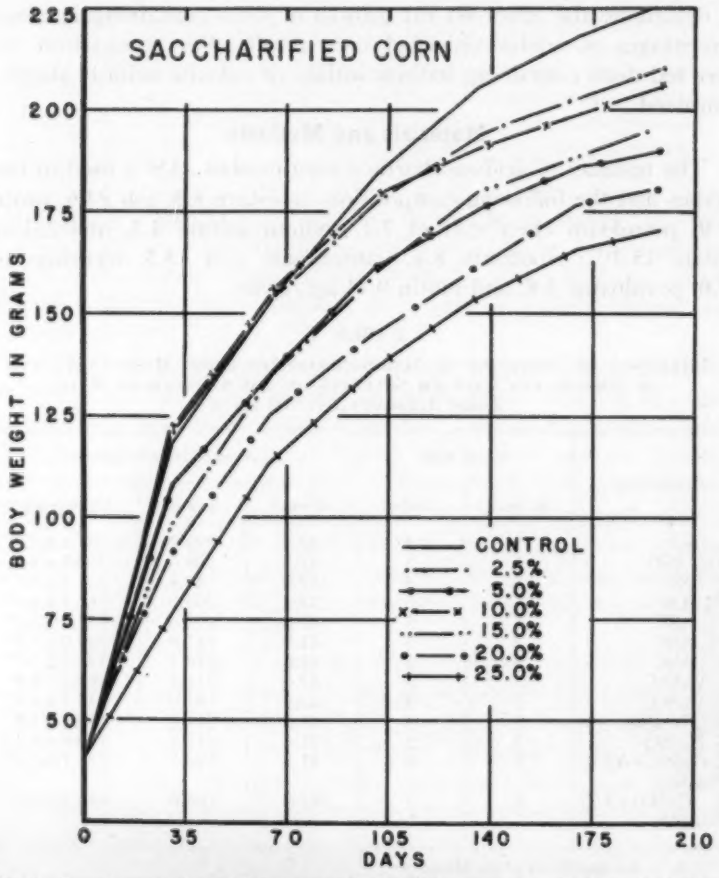


FIG. 1. Growth curves of rats fed diets containing increasing percentages of acid-saccharified corn.

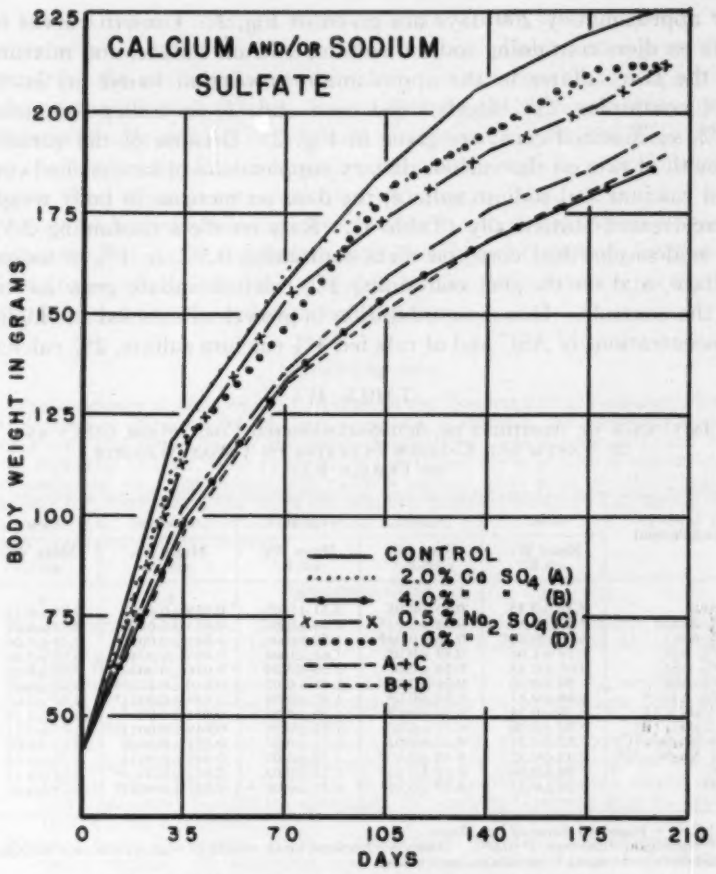


FIG. 2. Growth curves of rats fed diets containing sodium sulfate or calcium sulfate singly and combined.

were also made on the consistency of the droppings. On termination of the experiment all surviving rats were killed and subjected to gross postmortem examination. The liver, spleen, kidneys, heart, and adrenals from each rat were weighed, and sections of all organs were saved for histopathological examination. All tissues were fixed in formalin, and hematoxylin-eosin stained sections of the following were prepared, thyroid, heart, lung, liver, stomach, intestine, kidney, adrenals, spleen, pancreas, ovaries, and uninary bladder.

Results and Discussion

The effects on growth of continued oral ingestion of diets containing 2.5 to 25% of acid-saccharified corn (ASC) in the basal diet of rats

for approximately 200 days are given in Fig. 1. Growth curves for rats on diets containing sodium sulfate, calcium sulfate, and mixtures of the two sulfates in the approximate proportion found (*a*) in the diet containing 25% saccharified corn and (*b*) in a diet containing 15% saccharified corn, are given in Fig. 2. Because of the variable growth of rats on the various dietary supplements of saccharified corn and calcium and sodium sulfate, the data on increase in body weight were treated statistically (Table I). Rats on diets containing 2.5% of acid-saccharified corn, on diets containing 0.5% or 1% of sodium sulfate, and on the diet containing 2% calcium sulfate grew as well as the controls. However, inhibition in growth of rats fed the higher concentrations of ASC and of rats fed 4% calcium sulfate, 2% calcium

TABLE II

INFLUENCE OF ADDITIONS OF ACID-SACCHARIFIED CORN MASH (ASC) AND OF SODIUM AND CALCIUM SULFATES ON ORGAN WEIGHTS OF FEMALE RATS

Supplement	Liver	Spleen	Kidneys	Adrenals	Heart
	Mean Wt. ±S.E.[1]	Mean Wt. ±S.E.[1]	Mean Wt. ±S.E.[1]	Mean Wt. ±S.E.[1]	Mean Wt. ±S.E.[1]
	g.	*g.*	*g.*	*g.*	*g.*
Control	8.38 ±0.35	0.55 ±0.05	1.51 ±0.05	0.059 ±0.0042	0.85 ±0.12
2.5% ASC	7.60 ±0.40	0.38 ±0.02*	1.44 ±0.04	0.053 ±0.0036	0.72 ±0.07
5.0% ASC	6.70 ±0.20*	0.38 ±0.02*	1.39 ±0.04	0.041 ±0.0037*	0.73 ±0.06
10.0% ASC	8.74 ±1.06	0.47 ±0.05	1.65 ±0.04	0.056 ±0.0041	0.78 ±0.06
15.0% ASC	8.07 ±0.43	0.54 ±0.05	1.56 ±0.04	0.046 ±0.0030*	0.76 ±0.09
20.0% ASC	9.30 ±0.76	0.51 ±0.03	1.58 ±0.05	0.040 ±0.0023*	0.72 ±0.07
25.0% ASC	8.48 ±0.27	0.52 ±0.03	1.47 ±0.04	0.038 ±0.0031*	0.69 ±0.07
2% $CaSO_4$ (A)	8.10 ±0.58	0.44 ±0.03	1.56 ±0.07	0.054 ±0.0017	0.77 ±0.16
4% $CaSO_4$ (B)	8.92 ±0.90	0.57 ±0.02	1.53 ±0.11	0.048 ±0.0043	0.71 ±0.11
0.5% Na_2SO_4 (C)	7.72 ±0.51	0.44 ±0.04	1.51 ±0.03	0.054 ±0.0046	0.72 ±0.05
1.0% Na_2SO_4 (D)	7.33 ±0.32	0.40 ±0.02*	1.59 ±0.02	0.049 ±0.0035	0.76 ±0.03
A+C	7.96 ±0.40	0.42 ±0.04	1.51 ±0.03	0.042 ±0.0028*	0.73 ±0.13
B+D	9.24 ±0.51	0.49 ±0.02	1.49 ±0.06	0.037 ±0.0032*	0.72 ±0.02

[1] S.E. = Standard Error of the Mean.
* Significant difference, P (0.05). Difference between mean weight of organs from rats on experimental diets and organs from rats on control diet.

sulfate + 0.5% sodium sulfate, or 4% calcium sulfate + 1% sodium sulfate was statistically significant. The inhibition in growth with diets containing 5% or more of ASC was undoubtedly due to the sodium and calcium sulfate, since a mixture of 2% calcium sulfate and 0.5% sodium sulfate showed a similar inhibition in growth.

With diets containing 10, 15, 20, and 25% of acid-saccharified corn, 1% of sodium sulfate, and 4% of calcium sulfate + 1% of sodium sulfate, the droppings were consistently softer than those of rats on the other concentrations of the dietary supplements, or of the controls. This observation suggests a slight cathartic action of the diet due to the presence of sulfates. Food consumption of all rats in the various groups was not significantly different from that of the controls on the basic diet alone.

Table II shows average organ weights of rats in each group, together with the standard errors of the means. The t-test was used in comparing the mean values obtained on the experimental groups with those of the controls. With few exceptions the adrenals were most consistently affected. The adrenals of rats fed 15, 20, and 25% ASC and the two levels of calcium sulfate + sodium sulfate were definitely smaller than those of rats in the control group.

Since no histopathological lesions were found in any of the visceral organs studied, it is concluded that possibly the growth inhibitory effect (toxic effect) of ASC was due to the mild laxative action of sulfates present in the acid-saccharified corn.

Acknowledgments

The assistance of Dr. Lelland J. Rather of the Department of Pathology, Stanford University Medical School, San Francisco, California, for histopathological examination and evaluation of the tissue of rats from these experiments, and Mr. E. K. Doxtader and Mr. Paul V. Marsh of this laboratory is gratefully appreciated. The analytical data for sodium and calcium sulfate were made by the Physicochemical and Analytical Division of the Western Regional Research Laboratory and the sample and other analytical data were supplied by the Northern Regional Research Laboratory of this Bureau.

Literature Cited

1. Bauernfeind, J. C., and Boruff, C. S. Fermentation by-products as animal feeds. Am. Miller **72**(1): 182–183, 198 (1944); Ibid. **72**(2): 53–54, 71 (1944); Ibid. **72**(3): 50–51, 64–65 (1944).
2. Bauernfeind, J. C., Garey, J. C., Baumgarten, W., Stone, L., and Boruff, C. S. Nutrient content of alcohol fermentation by-products from corn. Ind. Eng. Chem. **36**: 76–78 (1944).
3. Fairbanks, B. W., Krider, J. L., and Carroll, W. E. Distillers' by-products in swine rations. I. Creep-feeding and growing-fattening rations. J. Animal Sci. **3**: 29–40 (1944); III. Dried corn distillers' solubles, alfalfa meal, and crystalline B-vitamins compared for growing-fattening pigs in drylot. Ibid. **4**: 420–429 (1945).
4. Krider, J. L., Fairbanks, B. W., Carroll, W. E. Distillers' by-products in swine rations. II. Lactation and growing-fattening rations. J. Animal Sci. **3**: 107–119 (1944).
5. Synold, R. E., Carrick, C. W., Roberts, R. E., and Hauge, S. M. Distillers' dried solubles as a vitamin supplement in chick rations. Poultry Sci. **22**: 323–329, 336 (1943).

THE AMINO ACIDS OF THE WATER EXTRACT OF FLOUR [1]

BETTY SULLIVAN [2] and W. E. PAYNE [3]

ABSTRACT

The following free amino acids were identified by filter paper chromatography in the water extract of a first clear flour: aspartic, glutamic, serine, glycine, tyrosine, lysine, arginine, histidine, threonine, alanine, methionine (as sulfoxide), proline, valine, phenyl alanine, and the leucines. Cystine (as cysteic acid) could be demonstrated only in the hydrolyzed water extract. Asparagine and glutamine also were found.

The same amino acids were found in the hydrolyzed soluble proteins precipitated by methanol and/or heat.

No significant differences in the position, size, and intensity of the spots were noted in either the water extract or the hydrolyzed soluble proteins from the same flour that had been heavily treated with nitrogen trichloride.

The purpose of work currently in progress is to determine if the valuable filter paper chromatography technique of Consden, Gordon and Martin (2) reveals any differences in the amino acids obtained from unoxidized and oxidized flours. Although it would be expected that such differences, if any, would be most evident in the hydrolyzed glutens, it was of interest to investigate first the water-soluble fractions of flour. This was of special interest because, although small amounts of amino nitrogen have been found in flour extracts by many investigators (1), no identification of specific amino acids present in the water-soluble extract of flour has been reported.

Materials and Methods

A spring wheat, first clear flour, analyzing 0.69% ash and 15.7% protein (14.0% moisture basis), was employed in all these experiments. Nitrogen trichloride treatment of the flour was carried out in 1-lb. lots in a laboratory bleacher at a level of 30 g. per hundred pounds.

Five hundred grams of flour were extracted with 1000 ml. of distilled water in a Waring Blendor. The extract was centrifuged and the soluble proteins precipitated from the clear water centrifugate by either methanol or heat (3 minutes at 97°C.). The solution obtained by centrifuging the precipitated water-soluble proteins was concentrated under vacuum and then divided into two equal lots.

[1] Manuscript received December 22, 1950.
[2] Russell-Miller Milling Company, Minneapolis, Minnesota.
[3] Present address: General Mills, Keokuk, Iowa.

One portion was hydrolyzed and chromatograms were obtained on both the hydrolyzed and unhydrolyzed portions.

All hydrolyses were done by refluxing with 20% hydrochloric acid for 20 hours after which the hydrolysates were filtered and evaporated to near dryness on a steam bath, taken up in distilled water, and then evaporated to complete dryness. When ready for use, the hydrolysates were diluted to the desired volume. It was necessary to maintain the pH within ± 0.2 in order to obtain reproducible results. One drop of solution (containing approximately 20 μg. of amino acids) was placed and dried on the filter paper. Two-dimensional chromatograms were obtained in these experiments by employing the equipment and general procedure described by Dent (3). Whatman No. 1 paper (18.5 $\times$ 22.5 in.) was employed. A solution of phenol saturated with water at 20°C. was always used for the first developing solvent. The trays in the bottom of this cabinet were filled with a 2-phase solution of phenol and water containing a few crystals of sodium cyanide. An evaporating dish containing 1% ammonium hydroxide was also placed in the bottom of the cabinet. Although a number of solvents was employed for the second development, the most satisfactory was a mixture of ethyl acetate, pyridine, and water, as employed by Jermyn and Isherwood (4) in the chromatographic identification of sugars. A solution of 0.2% ninhydrin in water-saturated butanol was used for the color development of the amino acid spots. The chromatograms were not subjected to heat at any time, drying of the paper being accomplished by air at room temperature.

A second chromatogram was made in the same cabinet and at the same time on all samples in order to show the presence of cystine. In these chromatograms, the hydrolysate spot on the filter paper was oxidized with a drop of 30% hydrogen peroxide before starting the run. In addition to producing a cysteic acid spot on the finished chromatogram, the oxidation also intensified the methionine sulfoxide spot found on the unoxidized chromatogram and produced a methionine sulfone spot.

Results and Discussion

With the exception of cystine, chromatographic positions of the amino acids from the hydrolyzed portion were identical with those found on the chromatograms of the unhydrolyzed portion, thereby demonstrating the existence of free amino acids in flour. The following amino acids were identified: aspartic, glutamic, serine, glycine, tyrosine, lysine, arginine, histidine, threonine, alanine, methionine (as sulfoxide), proline, valine, phenyl alanine, and the leucines (leucine and isoleucine occur together with the solvent used). Asparagine and

glutamine also were identified in the unhydrolyzed water extract. Cystine (as cysteic acid) was found only in the water extract that was hydrolyzed.

When the same procedure was followed using a flour heavily treated with nitrogen tricholoride, identical results were obtained.

The protein precipitates resulting from both the methanol and heat treatment of the water extracts of flour were hydrolyzed and chromatograms obtained from the hydrolysates. No significant differences were observed between these amino acid chromatograms nor between similar chromatograms obtained with the hydrolyzed precipitates resulting from methanol and heat coagulation of the water extracts of nitrogen trichloride treated flour. In all instances, the following amino acids were identified: aspartic, glutamic, serine, glycine, lysine, arginine, histidine, threonine, alanine, cystine (as cysteic acid), methionine (as methionine sulfoxide), proline, tyrosine, valine, the leucines, and phenyl alanine. No attempt was made to measure the amino acids quantitatively.

Literature Cited

1. Bailey, C. H. Non-protein Nitrogen in the Constituents of Wheat and Wheat Products. Reinhold, New York (1944), pp. 131–139.
2. Consden, R., Gordon, A. H., and Martin, A. J. P. Qualitative analysis of proteins: a partition chromatographic method using paper. Biochem. J. **38**: 224–232 (1944).
3. Dent, C. E. A study of the behavior of some sixty amino-acids and other ninhydrin-reacting substances on phenol-collidine filter paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. J. **43**: 169–180 (1948).
4. Jermyn, M. A., and Isherwood, F. A. Improved separation of sugars on the paper partition chromatogram. Biochem. J. **44**: 402–407 (1949).

A RAPID METHOD FOR DETERMINING THE VIABILITY OF BARLEY[1]

PAUL R. GUSTAFSON[2]

ABSTRACT

A new and rapid method has been developed for determining the viability of whole, unpearled barley kernels. The grain is treated with solutions of phosphoric acid, benzidine, and hydrogen peroxide containing sodium chloride, in the order named, and finally with Skellysolve B. Viable kernels remain uncolored or are colored clear yellow; dead kernels show appreciable orange coloration. Wherever the hull is broken or removed, a blue color appears.

The technique of the procedure requires only 35 minutes, in contrast to previous methods which require periods ranging from overnight to one week.

The standard germination test for barley requires a week for completion[3,4]. Stimulation of germination with hydrogen peroxide is used in some commercial laboratories; by this method, results are obtained overnight (1).

A method has been developed which gives results in 35 minutes. It is based on the use of benzidine to measure the decomposition of hydrogen peroxide (3) by substances in the barley hulls. Brücker (2) used benzidine and hydrogen peroxide to determine the germinability of cereal grains by measurement of the peroxidase activity of the cut kernels. A literature search has, however, revealed no previous application of these reagents for testing the viability of uncut, unpearled grain.

Materials and Methods

Reagents. (1) Phosphoric acid solution. To 1.5 ml. of 85% orthophosphoric acid sufficient water was added to make 100 ml. of solution.

(2) Benzidine solution. Five grams of C.P. grade benzidine were dissolved in 250 ml. of C.P. grade glacial acetic acid, and 750 ml. of water were added.

[1] Manuscript received September 18, 1950. Contribution from Osborne McMillan Elevator Co., Minneapolis, Minn.

[2] At present on active duty with the United States Army.

[3] Wilhoit Laboratories procedure: "100 kernels placed on paper towels, 10 × 15 in., rolled and wrapped in waxed paper and placed in germinator at a temperature of 20°C. First count after 3 days, final count after 6 or 7 days."

[4] Also, for long germination see Rules and Regulations under Federal Seed Act, Service and Regulatory Announcement No. 156—Reprinted with Amendments, July, 1950, pp. 14–23.

(3) Hydrogen peroxide solution. Commercial 35% hydrogen peroxide was diluted to 1% by addition of water (1 ml. diluted to 35 ml.). The solution must be stored in a dark bottle away from light and heat.

(4) Sodium chloride solution. One gram of sodium chloride was dissolved in 1 liter of water.

(5) Petroleum ether (Skellysolve B).

Distilled water was used in making all solutions. Unless tap water is fairly free of iron and manganese, a bleaching effect on the kernels may occur caused by ferrous iron (4).

All of the reagents are stable for at least one month. The benzidine solution should be protected from air as much as possible.

Procedure. One hundred kernels of barley were covered with 30 ml. of the phosphoric acid solution. At the end of 5 minutes (±1 minute), 10 ml. of the benzidine solution were added and the mixture was allowed to stand for 2 minutes (±0.5 minute). The barley was then removed and rinsed with distilled water. A sieve was used to facilitate the washing. The rinsed barley was placed in a freshly prepared mixture of 15 ml., each, of the hydrogen peroxide and sodium chloride solutions (3, 7, 8). Here the kernels were allowed to soak for at least 15 minutes (up to 40 minutes was found to be safe), then were rinsed with distilled water and placed in a Petri dish which contained sufficient Skellysolve B or F to cover them. At the end of 5 minutes, the Petri dish was placed on a light green or white background and the kernels were examined for color.

All kernels showing their original color or a true yellow color, with no tinge of orange, were counted as viable. If only a small pinpoint of orange color was observed at the germ end, with no orange color elsewhere, the kernel was considered to be viable. Dead kernels showed an orange tinge which usually covered from one-fourth to one-half of the kernel. Weathered kernels were sometimes dull, brownish orange when dead. An intense blue or purple color appeared where kernels were skinned or broken.

The samples may, if desired, be left in the Skellysolve B for several hours before examination. Samples can also be dried after the second rinse and kept up to 4 hours for subsequent examination; they must be placed in Skellysolve B before the count is made. The procedure should not, however, be interrupted before the second rinse, or the proper color may not develop.

The treatment with phosphoric acid is required for subsequent development of the yellow or orange color. Sulfuric acid (at 0.1% conc. or at 0.1% of the phosphoric acid conc. or less), but not acetic acid, may be used in place of phosphoric acid. Sodium chloride

accelerates the appearance of the orange color and intensifies the blue color which appears in damaged areas. Barley which has been stored for two years or longer becomes orange colored very slowly or not at all if the treatment with Skellysolve is omitted.

Check Procedures. Germinability of the samples studied was checked by:

(1) A blotter test carried out in a Mangelsdorf Germinator at 68°F. for one week.

(2) Overnight germination with hydrogen peroxide (1), carried out in commercial laboratories.

Statistical Analysis. Standard deviations and correlation coefficients between the results of the blotter method and the hydrogen peroxide method were computed (5).

Results and Discussion

The barley samples studied range in germination from 7 to 98%. Typical data are compared in Table I with results obtained on portions of the same samples by the two check methods. For the germination range 75 to 98%, the correlation coefficient between the

TABLE I

COMPARISON OF GERMINATION TESTS ON BARLEY BY THE BENZIDINE METHOD WITH THE BLOTTER AND HYDROGEN PEROXIDE METHODS [1]

Number of different samples	Germination			Standard deviation benzidine method
	Blotter method	Benzidine method	Hydrogen peroxide method	
	%	%	%	
22	98	98.2	97.8	0.4
23	96	96.0	96.0	2.0
29	94	95.1	95.2	2.3
10	90	91.3	91.3	2.6
15	88	90.4	91.9	4.3
12	85	85.4	88.8	4.5
10	83	83.3	84.7	2.7
6	80	79.0	80.8	4.1
1	78	85.0	78.0	—
5	76	79.6	80.8	4.1
2	75	76.5	74.5	—
1	60	66.0	57.0	—
1	57	64.0	45.0	—
1	54	57.0	53.0	—
1	38	43.0	42.0	—
1	32	41.0	—	—
1	28	10.0	21.0	—
1	14	21.0	17.0	—
1	13	7.0	11.0	—

[1] All samples with a germination value of 60% or less were tested in triplicate, excepting the last sample in the table which was tested in duplicate.

results of the Benzidine method and the average of the Blotter and Hydrogen Peroxide methods is 0.92. Three commercial laboratories furnished barley samples of unknown germination values. Results on these samples obtained by the benzidine method compared to the standard method showed the benzidine method to be 1 to 3 days more rapid and comparable in accuracy.

The advantage of the method lies in its applicability to uncut, unpearled grain, and in the short time required to obtain final results.

It has been found that by varying the concentration of the acetic and phosphoric acid the color can be increased or decreased. Higher concentrations of acetic acid produce a greater orange color while higher concentrations of phosphoric acid produce a greater yellow color. The test is more sensitive on intermediate samples (germination, 80–90%) if a higher concentration of acetic acid is used. Hard kernels which are slow to soak up the dye require an additional 1 minute of soak time.

Acknowledgments

The encouragement of Mr. K. Nold and of the Osborne McMillan Elevator Co., including permission to perform these experiments while in their employ, is gratefully acknowledged. Gratitude is also expressed to Mr. Don Bruce of the Minneapolis Grain Exchange for his interest in the study, the Atwood-Larson Co., the C. W. Ingman Laboratories and the A. D. Wilhoit Laboratories, all of Minneapolis, Minnesota, for cooperating on the hydrogen peroxide test method and for information on the overnight and blotter procedures.

Literature Cited

1. Bishop, L. R. Memorandum on barley germination. J. Inst. Brewing **50**: 166–185 (1944).
2. Brücker, H. Eine Schnellmethode zur Bestimmung der Keimfähigkeit von Samen. Physiol. Plantarum **1**: 343–358 (1948).
3. Hill, G. A., and Kelley, L. Organic Chemistry, p. 151. The Blackstone Co., Philadelphia, Penn. (1943).
4. Mummery, C. S. Effect of ferrous iron on hydrogen peroxide. J. Soc. Chem. Ind. **32**: 889–893 (1913). See also Chem. Abstracts **8**: 284.
5. Paterson, D. D. Statistical Technique in Agricultural Research, pp. 19–31, and Chapter V, pp. 106–129. McGraw Hill, New York (1939).
6. Snell, F. D., and Snell, C. T. Colorimetric Methods of Analysis, p. 541. D. Van Nostrand Co., New York (1936).
7. Stratton, R. C., Ficklen, J. B., and Hough, W. A. Colorimetric determination of traces of manganese and chlorine with benzine. Ind. Eng. Chem. (Anal. Ed.) **4**: 2 (1932).

BOOK REVIEWS

Advances in Agronomy. Vol. II. Edited by A. G. Norman. 407 pp. Academic Press Inc. New York, New York. 1950. Price $7.50.

The second volume of the series contains nine chapters, five dealing with agronomic crops and four with soils. Like Vol. I the subject matter is well organized and well documented with references.

The first chapter, a treatise on cotton, was prepared under the direction of Dr. J. E. Adams of Texas A. & M. College and comprises 74 pages. It includes eight sections exclusive of the introduction by Dr. Adams, which deal with all phases of the crop and its production. Included are sections on the competitive position of cotton among fibers, by M. K. Thorne, Jr., University of Mississippi; the physiology of the plant, by F. M. Eaton; diseases of cotton, by John T. Presley; insect pests by J. C. Gaines; improvement in production practices, by William E. Meek and Harris P. Smith; ginning practices, by Charles A. Bennett; fiber properties and their significance, by Henry D. Barker; and breeding and improvement, by T. R. Richmond.

The second chapter deals with soil nitrogen and was prepared by L. E. Ensminger, Alabama Agr. Exp. Station, and R. W. Pearson, U.S.D.A. The authors deal with such problems as factors affecting nitrogen content of soils; nature of organic nitrogen in soils; nitrogen transformations; effect of cropping practices on nitrogen level; nitrogen economy of eroded soils; and commercial nitrogen vs. barnyard manure and green manures. The chapter is well done and well be of special interest to agronomists, soil scientists and soil microbiologists.

The third chapter by J. E. Knott and O. A. Lorenz, University of California, deals with vegetable production. The chapter is prefaced by an interesting introduction which gives statistics on the amount and distribution of vegetable production in the United States. Since these crops are grown on high priced land fertilization, the use of improved high yielding varieties, and the control of insects and diseases become very important matters. These are discussed in detail as well as the use of heterosis in the production of F_1 seed. Other sections are devoted to growth control techniques, and labor saving devices in seeding, use of herbicides and harvesting machinery.

The fourth chapter on Prairie Soils of the Upper Mississippi Valley was written by Guy D. Smith, U.S.D.A., W. H. Allaway and F. F. Riecken, Iowa State College. The authors have had wide experience in the soils of this area are in a position to deal with them in an authoritative manner. They discuss the characteristics of a model prairie soil and variability of prairie soils as functions of soil forming factors such as biotic factors, climate, parent materials, time, and topography. The chapter closes with a discussion of crop yields and soil changes produced by cultivation. The chapter is of especial interest to those soil scientists who deal with soil genesis and classification.

Gilbert H. Ahlgren, Rutgers University, and R. F. Fuelleman, University of Illinois, have prepared an excellent chapter (V) on Ladino clover. They discuss in detail the history and distribution, characteristics, adaptation and management of the crop. Soil, lime, and fertilizer requirements receive consideration as do seeding practices, suitable companion crops and utilization of the crop. Since Ladino clover is widely used as a constituent of pasture mixtures and for hay and silage the chapter will be of especial interest to agronomists and stockmen.

Chapter VI, dealing with the control of soil water and authored by E. C. Childs and N. Colles-George, University of Cambridge, England, presents technical information that will be primarily of value to soil physicists and hydraulic engineers. The authors discuss the basic approach to the solution of moisture problems and the application of this to the solution of problems of irrigation and drainage.

The seventh chapter concerns the preservation and storage of forage crops and was written by R. B. Musgrove and W. K. Kennedy, Cornell University. It deals with the very practical problems of measurements of changes in quality during preservation and storage; the ensilage process and problems of silage making; the field-curing of hay and factors which influence its quality of hay; barn curing of hay

and operation of barn driers; and artificial drying by driers of various types. The chapter emphasizes that the feeding values of forage crops are at a maximum for a relatively short time during their development and that to retain their full value a system of handling, preservation and storage must be employed which removes the crop from the field rapidly and at the same time preserves it so that minimum loss of quality occurs.

Much of the coal mined in the United States is produced by the open pit method. In the eighth chapter Dr. Helmut Kohnke, Purdue University, deals with the problem of the reclamation of coal mine spoil banks. Materials in these spoils are very heterogenous, ranging widely in texture, reaction and topography. All are low in organic matter and nitrogen but otherwise the fertility is generally similar to natural soils. Methods of revegetation, using trees, pasture grasses and wildlife plantings, are suggested. The land use capabilities of these spoils is dependent on their properties as they relate to texture, reaction and topography.

The ninth and last chapter of the volume deals with irrigated pastures and is authored by Wesley Keller, U.S.D.A., and Maurice L. Peterson, California Agr. Exp. Station. The importance of the character of the soil in relation to management is pointed out and methods of establishment of pastures for irrigation discussed. Stress is laid on pasture management under irrigation. Sections on the economy of pastures and pastures in relation to other sources of feed round out the excellent treatment of the subject.

The volume as a whole is very well done. It should be of wide interest to not only agronomists and soil scientists but to growers as well. Chapters on cotton, vegetable production, ladino clover, preservation and storage of forage crops, and irrigated pastures should prove of much practical value to growers and others interested in production.

Dr. C. O. Rost
Division of Soils
University of Minnesota
St. Paul 1, Minnesota

The Biochemistry of Nucleic Acids. J. N. Davidson D.Sc., M.D., Gardiner Professor of Physiological Chemistry in the University of Glascow. London: Methuen & Co. Ltd. New York: John Wiley & Sons, Inc. Price $1.75.

Professor Davidson prefaces his book with the apology that it is not "an exhaustive treatment of the biochemistry of nucleic acids (but) is intended rather to provide an elementary outline of the main features of the nucleic acids and nucleoproteins for the benefit of students of biochemistry, of chemists who wish to know something about the biological aspects of the subject, and of biologists who wish to learn a little about the chemical aspects."

The apology is unnecessary for he has not only accomplished his stated objective but has also assembled and organized fundamental facts and observations from a variety of sources such that the book will also have very real value to those actually working in the field of the nucleic acids. He has not attempted to cite all of the original literature in this rapidly expanding field, but he has given ample original references for the key observations in addition to citing review articles which more intensively cover restricted aspects of the field.

After a brief historical introduction Davidson discusses the hydrolysis products of nucleic acids—the purines, pyrimidines and sugars, then the nucleosides and nucleotides. At this point he briefly introduces the reader to the chromatographic technique for the separation of these hydrolysis products. From here he goes on to a discussion of the properties and proposed structures of the polynucleotides and nucleoproteins and then to a consideration of the enzymes that act on nucleic acids and their derivatives. His discussion of the ultraviolet absorption of nucleic acids, and of the applications that have been made for analytical purposes, points out the potentialities of this approach but would be stronger if the limitations were more adequately covered. The chemical and histochemical methods for detection and estimation of the nucleic acids are summarized and briefly discussed in two chapters.

Up to this point Davidson has set forth the fundamental facts which are necessary to evaluate the observations on biological systems which he reports in the last half of the book. In this last part he presents in readable form the available observations on the nucleic acid content of tissues and on the composition of the cell cytoplasm and nucleus. He then goes on to a discussion of the metabolism and

biosynthesis of nucleic acids including the pertinent and fascinating information obtained from spectrophotometric and isotopic investigations. Finally he concludes with a summary of the work on the nucleic acids of bacteria and viruses.

CYRUS P. BARNUM, JR.
Department of Physiological Chemistry
Medical School
University of Minnesota
Minneapolis 14, Minnesota

Methods of Vitamin Assay. The Association of Vitamin Chemists, Inc. 301 pp. Interscience Publishers, Inc., New York, 1951. Price: $5.50.

The present edition should achieve the wide acceptance and general usage which was met by the First Edition of Methods of Vitamin Assay (1947), inasmuch as the current volume augments the first edition and reflects increasing experience and recent improvements in vitamin methodology.

The methods presented in the first edition have been revised to incorporate pertinent information from the current literature as well as modifications and changes in procedure suggested by the many interested users of the book. Besides improved assay methods for vitamin A, carotene, thiamine, riboflavin, niacin and ascorbic acid, the present edition includes procedures for several members of the vitamin B complex for which methods were not described in the first edition, namely pantothenic acid, pyridoxine, folic acid, biotin, and vitamin B_{12}. A chemical analysis for niacin is also described.

Because a number of vitamins are determined microbiologically, a complete chapter is devoted to Microbiological Assay Technics. This should prove very helpful to analysts involved in this type of vitamin assay.

A discussion and extensive bibliography concerning vitamins D, E, and K and p-aminobenzoic acid, inositol, and choline may be found in one of the concluding chapters of the book. In addition, the lists of recent literature related to each subject and vitamin discussed make this work a valuable reference volume.

MAXWELL L. COOLEY
Products Control Department
General Mills, Inc.
Minneapolis 14, Minnesota

Essentials of Nutrition. By Henry C. Sherman and Caroline Sherman Lanford, 1951. Third edition, ix + 454 pp. Macmillan Co., New York. Price $4.25.

This book is intended primarily for those who are being exposed to the science of nutrition for the first time. It constitutes a simple, logical treatment of the fundamentals of nutrition and "assumes no prerequisite of training in science." The complete absence of chemical formulae is evidence of the conscientious attempt that was made to adhere to this principle. A brief consideration of digestion and metabolism serves to introduce the main subject matter which appears in a sequence that closely parallels the chronological development of the science of nutrition, namely, (1) the energy aspects of nutrition, (2) the proteins and their amino acids, (3) the mineral elements, and (4) the vitamins. The chapters are so written, however, that they may be studied in any sequence desired. Figures and illustrations have been well chosen and serve to demonstrate in an impressive manner the various principles under consideration.

One cannot help but be impressed by the emphasis which is placed on the practical application of nutritional knowledge to the health and well-being of the individual. Considerable prominence is given to a consideration of such factors as the quantitative requirements for nutrients (with particular stress on the wide zone of beneficial increase between the minimal-adequate and the long-run optimum level of intake in the case of such nutrients as calcium, ascorbic acid, riboflavin, and vitamin A), their distribution in nature, and their susceptibility to destruction. In addition, whole chapters are devoted to such topics as: "How to Meet the Energy Need"; "How to Meet the Need for Protein"; "Food Costs and Values"; and "How to Make Nutritional Knowledge More Effective."

Valuable adjuncts to the text include a glossary of the more technical terms, a comprehensive compilation of the composition and nutritive value of various food

items, and recommended dietary allowances (as of 1948). Practical exercises and suggested readings at the end of each chapter not only enable the student to test his understanding of the text but also serves to stimulate further interest in topics which, of necessity, have been briefly presented.

To one who has been exposed to the more advanced aspects of nutrition, errors of omission are not difficult to find. In an elementary text of this nature, however, it may be difficult to justify such a criticism. With this reservation in mind, this reviewer would, nevertheless, question the wisdom of omitting such facts of which the following are examples: 1) the tryptophane-niacin relationship as a possible explanation for the pellaga-preventive properties of milk which is low in niacin, and 2) biological methods for evaluating the nutritive value of proteins in the section which bears the title: "How may the nutritive value of a protein be assessed."

Emphasizing as it does the application of the science of nutrition to every day living, this book should provide a substantial contribution to the knowledge required by those who will be concerned with the maintenance of the health and efficiency of our community.

IRVIN E. LIENER
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minnesota

Introduction to Agricultural Biochemistry. By R. Adams Dutcher, Clifford O. Jensen, and Paul M. Althouse, 1951. xii + 502 pp. John Wiley and Sons, Inc., New York. Price $6.00.

This book has been written to replace the "Introduction to Agricultural Biochemistry" produced by Dutcher and Haley in 1932. Much information has been gathered in the past nineteen years and this reviewer has been pleasantly surprised at the wealth of material incorporated in the text. The book is primarily designed for students at the undergraduate level in agriculture and in other fields which touch upon the chemistry of plant and animal life. After the first part, which deals with the traditional material of general biochemistry, the authors treat the chemical changes in plants and in animals. The material on the mineral and the protein metabolism and their role in nutrition has been considerably expanded and several complicated processes have been clearly presented without sacrifice of accuracy. The chapters on farm chemurgy and pesticides are unique in a text of this sort. This is one of the few books of its kind which deals with the biochemical processes in both plants and animals.

To readers of this journal who may want information on the processes involved in plant and animal life this book should serve as a very readable survey.

W. M. SANDSTROM
Division of Agricultural Biochemistry
University of Minnesota
St. Paul, Minnesota

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for *Cereal Chemistry*.

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

Sterling-Winthrop Research Institute serving Sterwin Chemicals Inc.

Sterwin

HELPS YOU PRODUCE BETTER FOOD PRODUCTS

Easily . . . Accurately . . . Economically

STERWIN CHEMICALS, backed by the extensive manufacturing and research facilities of the entire Sterling Drug organization, is continually striving to supply food processors with modern products and methods that will aid in the production of *better* food products *easily*, *accurately* and *economically*.

Contributions to the Food Industry by Sterwin products have been many. Outstanding among them are the following famous products . . . each a *first* in its field:

FIRST — BREAD-ENRICHMENT TABLETS

B-E-T-S® Enabled bakers to comply promptly with War Food Order No. 1. This tablet method has made enrichment in the bakery both practical and economical . . . saving bakers many millions of dollars.

FIRST — LIGNIN VANILLIN MADE IN U. S. AMERICA

ZIMCO® Pure Lignin Vanillin U.S.P. Made by world's largest Vanillin manufacturer. Preferred by leading flavor suppliers and manufacturers of confections, ice cream, baked goods, soft drinks and other foods.

FIRST — LOW-ASH FOOD ENRICHMENT MIXTURE

VEXTRAM® The original starch base enrichment mixture. Keeps market value of enriched flour up by keeping the ash content low. Free-flowing . . . uniform . . . stable.

FIRST — SYNTHETIC VITAMIN D_3

TRIDEE® The form of Vitamin D which has the greatest antirachitic value in poultry. D_3 is also used for fortification of evaporated milk and other food products.

FIRST — QUATERNARY AMMONIUM GERMICIDE

ROCCAL® A powerful germicide that may be used for every equipment sanitizing job throughout the Food Industry.

FIRST — WATER MISCIBLE, CRYSTALLINE VITAMIN D_2

DELTAXIN® The purest known form of Vitamin D_2. For fortification of milk, bread and other food products.

BRANCHES AND STOCK DEPOTS IN EVERY SECTION OF THE COUNTRY.

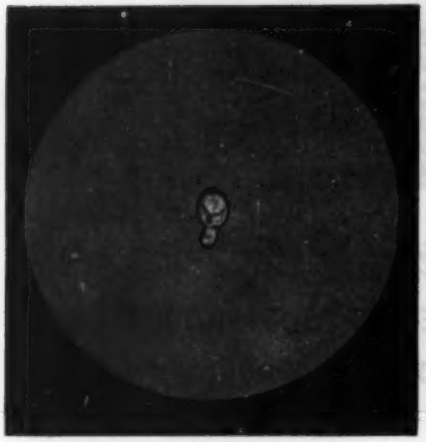
Sterwin Chemicals Inc.

Subsidiary of Sterling Drug Inc.

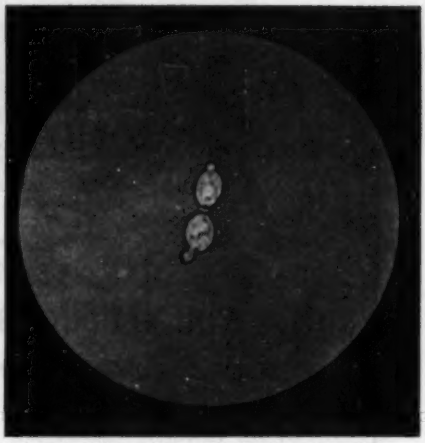
1450 BROADWAY, NEW YORK 18, NEW YORK

Pioneers in Food Enrichment

Distributor of the products formerly sold by Special-Markets-Industrial Division of Winthrop-Stearns Inc., and Vanillin Division of General Drug Company.



1. From this single, vigorous, budding yeast cell . . .



2. A strain of fine, active yeast is started . . . (1 hour)

LIKE COUNTING THE STARS IN THE HEAVENS. This colony counter aids skilled bacteriologists in controlling the quality of yeast. The scientific method is always used to produce Fleischmann's!

BABY YEAST outgrows its "clothes" but fast. Seed yeast is tiny for only a little while. From small glass flasks at the start, it requires ever larger containers. Some end up in immense stainless steel tanks.

EVEN THE CONTROLS ARE CONTROLLED. Constant watchfulness is the price of a uniform quality yeast. Here, a trained technician adjusts one of the heaters on the Kjeldahl apparatus used to determine the amount of nitrogen in yeast—one of the many controlling factors in yeast quality.

PROOF OF THE YEAST IS IN THE BAKING. Loaves look perfect, don't they? Here Floyd Schoonover, in charge of the Commercial Bakery Unit, scores bread for color, grain and texture, three of the many factors in the total score. Commercial type loaves are baked regularly as a final check.

A FAMILY

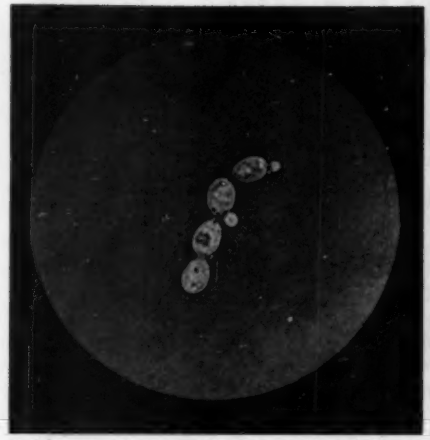
. . . of energetic yeast you bake better goods, increase profits!

• Your pound cake of Fleischmann's Yeast began as a single *microscopic* yeast cell. Selected for strength and vigor, it reproduced itself billions of times.

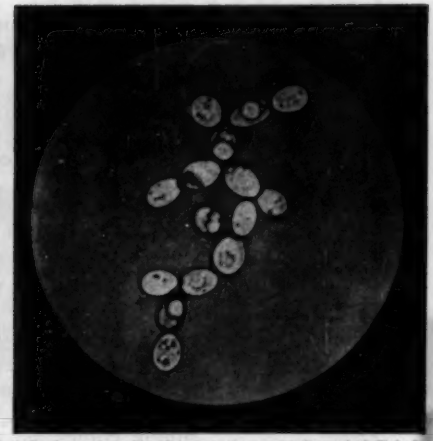
But the leavening ability of this family of billions would never be realized if its quality and uniformity were not closely supervised during growth.

Fleischmann scientists and technicians, working in the world's greatest center of yeast knowledge, have the facilities to control, produce and deliver to you, yeast that is *dependable*.

The uniform baking results you enjoy is the product of *more than 80 years* of Fleischmann research and development. When you use Fleischmann's Yeast, your baked goods have top flavor, quality and sales appeal . . . while your operating costs are reduced *and profits increased*.



3. Under supervision, growth is encouraged by ideal reproduction conditions . . . (2 hours)

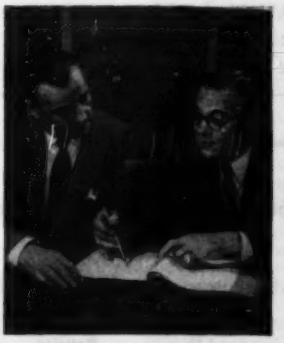


4. So that only uniformly energetic yeast cells go into Fleischmann's Pound Yeast. (3 hours)

PORTRAIT

cells that can help reduce costs and

Free Fleischmann service. You'll find Fleischmann men alert to help you with any bakery production or selling problems you may have. Call on them for expert help at any time. No obligation!



BAKERY PRODUCTION SERVICE. Your needs and problems are familiar to Fleischmann personnel. Mr. G. H. Ekstedt, right, Bakery Production Service manager, checks production costs and product quality for a baker customer.

FIND OUT WHY more bakers than ever say, "Use Fleischmann's Yeast for the finest fermentation. Keep quality and sales high with Fleischmann Service." Just call your Fleischmann man.

IN THE NEW BRIGHT BLUE-AND-WHITE WRAPPER. Famous for over 80 years. Bakers know there's no finer yeast in the world than Fleischmann's.

Make it better... sell more of it with

FLEISCHMANN'S YEAST

FERMENTATION IS OUR BUSINESS

Member

NOTICE TO SUBSCRIBERS

The increased cost of publishing Cereal Chemistry has made it necessary to raise subscription rates. The following rates will be effective as of July 15, 1951.

CEREAL CHEMISTRY

Single copies (current year) ..... $ 2.50
(Foreign postage 10 cents extra)
Annual subscriptions $11.00
(Foreign postage 50 cents extra)

Back issues:

Single copies ..... $ 3.00
Volumes $15.00

TRANSACTIONS

Single copies (current year) ..... $ 1.25
Annual subscriptions ..... $ 2.00
(Foreign postage 10 cents extra)

Back issues:

Single copies ..... $ 1.50
Volumes (two issues) ..... $ 2.50

SPECIAL COMBINATION OFFER: Cereal Chemistry and Transactions when ordered together, $12.00 (foreign postage 60 cents extra).

Technical Facts about **LARVACIDE** *for the Cereal Chemist*

(CHLORPICRIN)

WHAT IT IS—Larvacide (chlorpicrin), also known as tear gas, is a homogeneous liquid which, upon exposure to air, volatilizes rapidly.

PHYSICAL CONSTANTS

9¼ fluid oz.	1 lb.	Density	1.66 at 20°C.
1 pint	1¼ lbs.	Boiling point	112°C.
1 quart	3½ lbs.	Melting point	−69°C.
1 gallon	14 lbs.	Vapor pressure	18.2 mm. at 20°C.

USES OF LARVACIDE—Larvacide is in general use in fumigation of grain, cereals, rice, flour, feed, seeds, tobacco, cigars, soils, dried fruits, nuts, rugs, furniture and other products and articles subject to insect attack. It works without damage or after-effect. At low concentrations it is also fatal to rats and mice. Gives tear gas safety warning!

HOW IT IS SHIPPED—Larvacide is shipped in liquid form (not under pressure) in steel cylinders 25 to 180 lbs. net, and in 1 lb. bottles, each in safety can, 12 to corrugated carton.

For complete information on Larvacide for control of profit-eating insects and rodents, kindly address your request to Dept. CC751.

INNIS, SPEIDEN & CO., Inc.

117 LIBERTY STREET • NEW YORK 6, N.Y.

BOSTON CHICAGO CINCINNATI CLEVELAND PHILADELPHIA LOS ANGELES OMAHA SAN FRANCISCO

Subsidiary E. S. BROWNING CO., INC., San Francisco—Los Angeles

NOW...GREATER STABILITY OF THIAMINE IN FLOUR WITH

Thiamine Mononitrate Merck

CEREAL chemists have welcomed with enthusiasm the introduction of Thiamine Mononitrate, now available in the full line of Merck Vitamin Mixtures for Flour Enrichment.

Thiamine Mononitrate is a more stable form of thiamine, assures a more complete retention of vitamin B_1 content in enriched flour—even under adverse conditions of temperature and humidity during shipping and storage.

Leading cereal chemistry laboratories participated in the thorough investigation of Thiamine Mononitrate in enriched flour. Since then, extensive commercial experience and rigorous field tests have confirmed that Thiamine Mononitrate represents a signal improvement in flour enrichment; that it offers a degree of protection hitherto not available.

Each ounce contains 369 milligrams Thiamine Mononitrate

NO CHANGE IN LABELING REQUIRED

Thiamine Mononitrate meets every requirement for thiamine under the Federal Standards of Identity for enriched flour. No need for change in the labeling on bags or packages to comply with Federal requirements when you use these improved Merck Vitamin Mixtures.

Each ounce contains 738 milligrams Thiamine Mononitrate

MERCK & CO., INC.
Manufacturing Chemists
RAHWAY, NEW JERSEY
In Canada: MERCK & CO. Limited—Montreal

SHORTENINGS YOU CAN DEPEND UPON

PRIMEX B&C

An all-hydrogenated vegetable oil shortening of exceptional stability. Excellent for all deep frying purposes. Preferred by biscuit and cracker bakers, manufacturers of prepared biscuit, pie crust, and doughnut flours, and makers of other food products where rancidity troubles are to be avoided.

SWEETEX

The "High-Ratio" shortening. Especially designed to permit bakers to produce "High-Ratio" cakes, icings, and sweet yeast goods with superior eating and keeping qualities.

NUTEX

An all-hydrogenated vegetable oil shortening combining the exceptional stability of Primex B&C and the "High-Ratio" properties of Sweetex. A top quality shortening manufactured for use in prepared cake, yeast raised doughnuts and sweet yeast dough, and icing mixes.

PRIMEX

The all-hydrogenated shortening "that sets the quality standard." A top grade shortening especially recommended for doughnut frying, for pies, cookies and bread, and for other shortening purposes.

PROCTER & GAMBLE

Branches and warehouses in principal cities

General Offices . . . CINCINNATI, OHIO

"Finest in the World"
for
FRAGMENT COUNT
and RODENT HAIR

Bausch & Lomb
Stereoscopic Wide Field
MICROSCOPE AKT-5

Equipped with
1x 3x 6x objectives
10x wide field eye pieces
Magnifications 10, 30 and 60
All other accessories in stock

Suggested by the Sanitation Committee for foreign matter detection, this AKT-5 Wide Field Microscope—watch-like in precision—dustproof in design—assures the quality performance which only Bausch & Lomb "know-how" can guarantee.

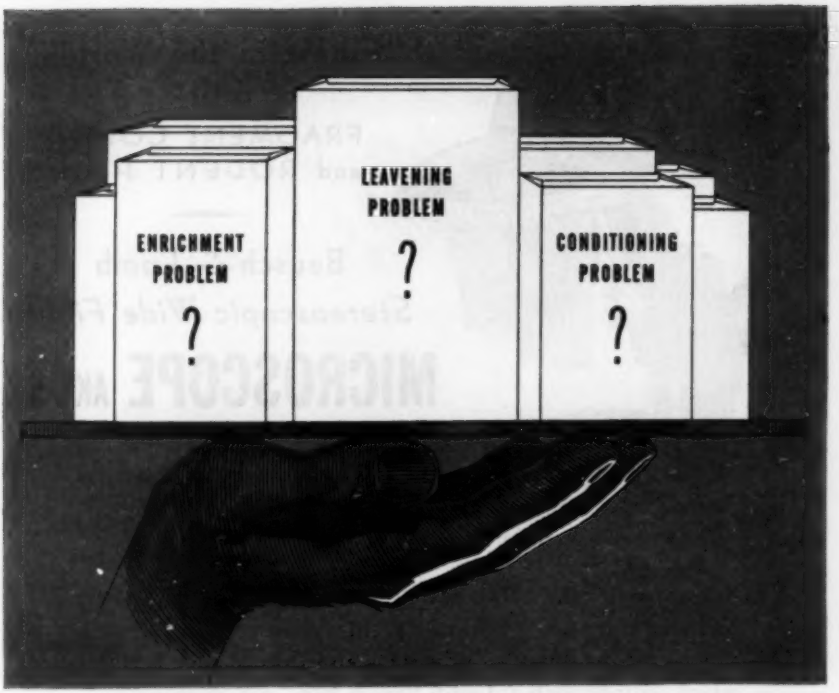
#31-33-11-01 Bausch & Lomb reflector illuminator with #31-36-45 rheostat. Jointed arm permits illumination from any angle. Equipped with blue daylight bulb.

We also supply Wildman Trap Flasks, 1,000 c.c. and 2,000 c.c. to be used in conjunction with this equipment.

Enquiries Invited. Write or wire today.

"IN SERVICE TO RESEARCH"

A. J. GRINER COMPANY
1827 McGee St. Kansas City 8, Mo.
Laboratory Apparatus • Chemicals



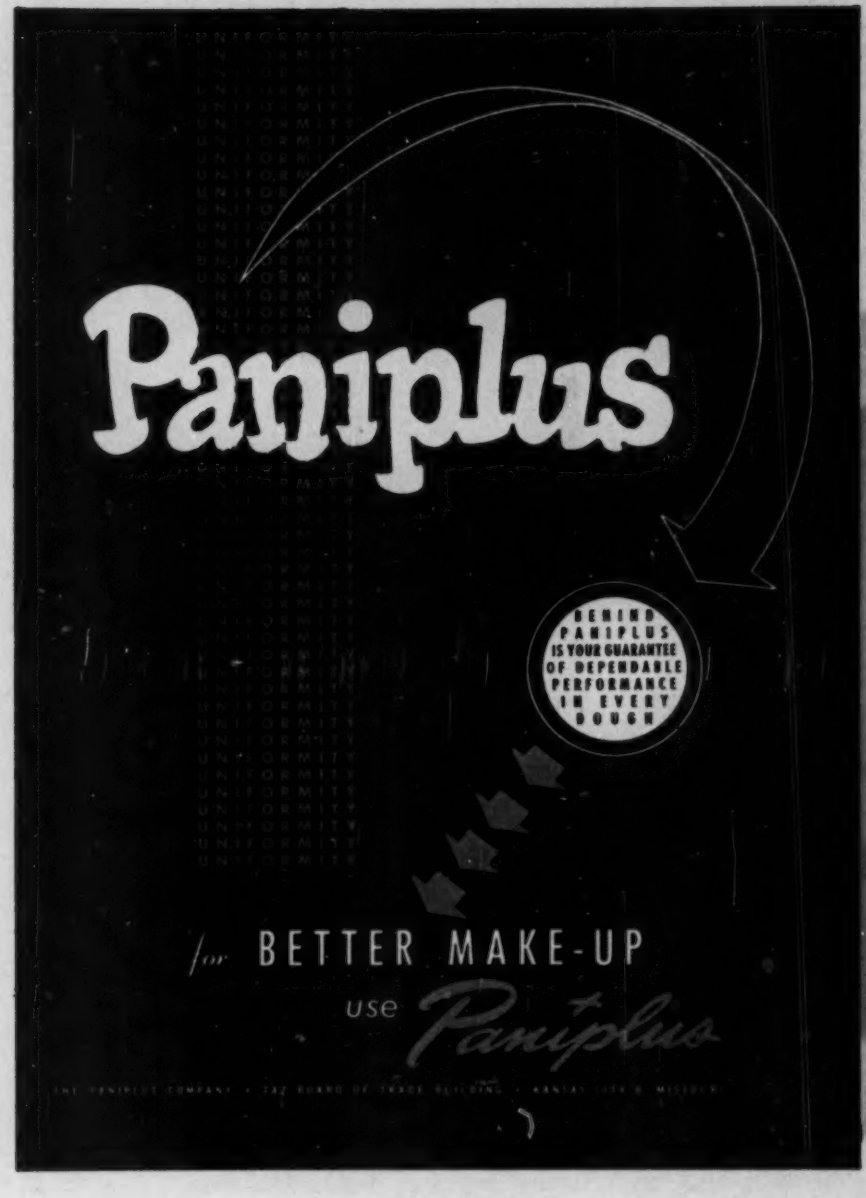
How You Benefit from Victor Experience

Producers of a wide variety of foods have discovered that it pays to check with Victor on product problems. Frequently the right answer is already in Victor's files. This is how you benefit from Victor's fifty and more years of experience in working on food production problems. Do you use phosphates for leavening, enrichment, conditioning? Do you have a special problem that may be solved with a phosphate? Bring your problem to Victor. We may already have the answer . . . or a clue to it. This is a part of Victor's accent on service to industry. Write today. There's no obligation, of course.

VICTOR CHEMICAL WORKS

VICTOR CHEMICAL WORKS

141 West Jackson Blvd. • Chicago 4, Illinois


Paniplus
BEHIND PANIPLUS IS YOUR GUARANTEE OF DEPENDABLE PERFORMANCE IN EVERY DOUGH
for BETTER MAKE-UP
use Paniplus

Service When It Counts

Every so often, we all need assistance and need it badly, to furnish the answers to many of our problems. In the Flour and Cereal Industry, N-A's Flour Service Division has been supplying practical, down-to-earth solutions to many of the industry's problems for over 25 years. Using its broad background of experience in the manufacture of time-tested products for maturing, bleaching, and enriching, the N-A Flour Service Division is ready to put its nation-wide service organization and extensive laboratory and research facilities at your service. They will work closely with your own staff and consultants to produce a concrete, individual solution to fit your particular needs.

For more details, call your nearest N-A Representative today and find out how this service can work profitably for you.

WALLACE & TIERNAN COMPANY, INC., AGENTS FOR

NOVADEL-AGENE

BELLEVILLE 9, NEW JERSEY

DYOX
for flour maturing
NOVADELOX
for a whiter, brighter flour
N-Richment-A
for uniform enrichment

NA-39*